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**14. ABSTRACT:** Macrophages are the first inflammatory cells to respond to asbestos fibers and we have shown that they are the predominant inflammatory cell in the asbestos-induced tumor, mesothelioma. We are beginning to unravel the role of the macrophage in this intractable tumor, using in vitro models of macrophages grown with mesothelioma cells, ex vivo models of human mesothelioma maintained as tumor fragment spheroids, and in 2 in vivo models of immunocompetent murine mesothelioma. In this first year, we have confirmed by immunohistochemistry and by flow cytometry of fresh tumor that macrophages are the main inflammatory cell in mesothelioma. We have shown that we can polarize macrophages to either an M1 (anti-tumor) or M2 (pro-tumor) phenotype and that M1-polarized macrophages (derived from a variety of sources including human mesothelioma) increase mesothelioma cell apoptosis and chemosensitivity. In our first study using an orthotopic murine mesothelioma model, the depletion of macrophages increased the mesothelioma response to chemotherapy. We aim to understand the role of the macrophage and how polarization or depletion of macrophages within the tumor can sensitize the mesothelioma cell to treatment.

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## I. INTRODUCTION

Inflammation is an early and consistent feature of mesothelioma [1, 2], an incurable tumor with a high level of resistance to chemotherapy [3]. Within this inflammatory milieu, we have discovered that there is a large population of macrophages, in a higher percentage than we have found in other thoracic malignancies. The macrophage is perhaps the earliest cell recruited to the asbestos fibers that are the cause of mesothelioma. After the tumor forms, macrophages are also, as we have found, a constant feature of mesothelioma. The association of macrophages appears thus to be a feature of mesothelioma during its initiation as well as its progression over four decades.

Macrophages, often known as tumor-associated macrophages, are found, albeit to a lesser extent, throughout many solid tumors. Increasingly, they are recognized as a key factor enhancing the malignant features of the tumor [4]. In separate tumors, a greater extent of macrophage infiltration in a tumor correlates with worse prognosis [5, 6]. Macrophages are now understood to have different phenotypes, directed by their cellular and cytokine environment [7]. The classic macrophage phenotype, termed M1 in analogy to the Th1 phenotype of lymphocytes, is directed to phagocytosis and anti-bacterial actions. This classic activation is seen in the pro-inflammatory environment, where LPS or interferons are prominent. More recently, an M2 phenotype was described that is directed by other cytokines, namely IL4, TGF beta. This alternatively activated macrophage is considered to be important at for the resolution of inflammation, dampening the inflammatory response and orchestrating a healing or tissue remodeling phase [8].

We wish to understand the mechanisms by which macrophages influence mesothelioma cells and whether we can manipulate the tumor-associated macrophages within the tumor by deletion or by alteration of the macrophage phenotype to sensitize tumors to apoptosis. To address these questions, we have several in vitro, ex vivo and in vivo approaches to investigate the mechanisms of macrophage-mesothelioma cell interactions. Our studies are designed to test the hypothesis that macrophages influence mesothelioma cell survival and can be manipulated to enhance mesothelioma cell apoptosis and response to chemotherapy.

## II. RESEARCH ACCOMPLISHMENTS

***Task 1. To determine the functional significance of macrophage phenotype on mesothelioma cell survival.***

**a. Elucidate the percentage of immune cells (CD45+) in human mesothelioma tumors and correlate immune cell infiltration with histopathologic subtype (months 1-6).**

Using tumor tissue microarrays of 71 mesothelioma tumors in the laboratory including new tumors obtained over the first year of funding, we have performed extensive immunohistochemical staining for immune cell populations and have correlated the macrophage population with the histologic subtype of mesothelioma. The intensity of staining of the cell population was assessed by digital analysis software (Aperio) to avoid investigator bias. The tumors were from patients who had not received chemotherapy;

interestingly, in other studies, there were no differences noted in those tumors from patients given prior chemotherapy. In mesothelioma, as in other tumors studied in the laboratory, inflammatory cells (CD45) make up a large percentage of the cells within the tumor (**Figure 1A**). This percentage is similar in each mesothelioma pathologic subtype. The macrophages are a large percentage of the inflammatory cell population (**Figure 1B**). It is also interesting that, in the few sarcomatous tumors studied so far (n=8), macrophages may constitute a higher percentage of the total inflammatory cell population than in epithelial or mixed tumors. If indeed macrophages act to support tumor survival, then a high percentage of macrophages in sarcomatous tumors could help explain the poor prognosis of this cell type. On the other hand, the ability to repolarize such macrophages might provide a useful therapeutic tool against such a recalcitrant histologic subtype of mesothelioma.

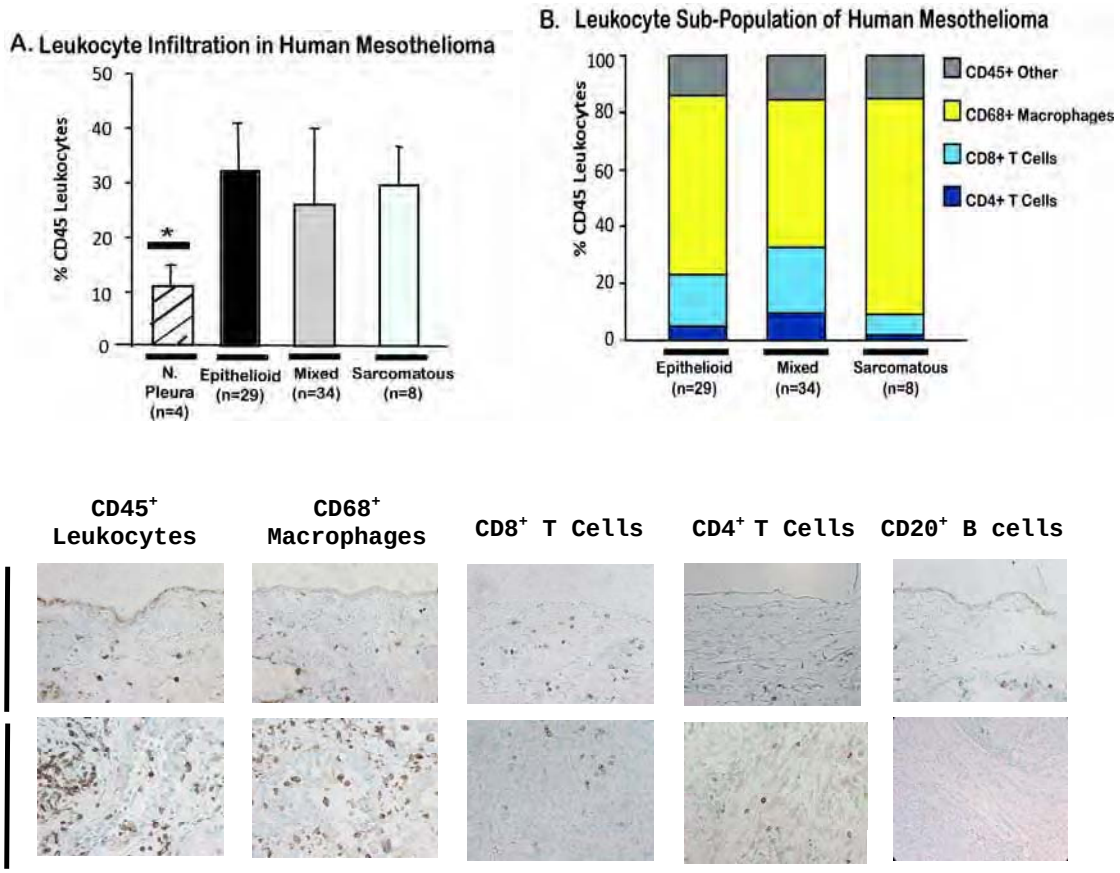


**Figure 1. Macrophages are a significant percentage of the inflammatory cell population in all mesothelioma histologic subtypes. A**: Leukocyte infiltration of human mesotheliomas was determined by histological analysis using $CD45^+$ staining. Tumor microarrays containing 71 mesothelioma tumors and 4 normal pleura samples were stained using anti-human $CD45^+$ primary antibody. Tumors were then grouped based on tumor subtype (epithelioid, mixed/biphasic, or sarcomatous.) Quantification of $CD45^+$ cells was conducted using Aperio Image analysis for membrane staining. Statistical significance was determined using ANOVA, where *$p<0.05$. **B**: Leukocyte sub-population was determined by histological analysis using anti- human $CD68^+$ macrophage, $CD8^+$ T cell, $CD4^+$ T cell staining on serial sections of human mesothelioma tumor microarrays. Quantification was conducted using Aperio Image analysis for membrane or nuclear staining. **C**: Immunohistochemistry of formalin-fixed normal human pleura (top) and mesothelioma (bottom). Tissues were stained for CD45+ leukocytes, CD68+ macrophages, CD8+ T cells, CD4+ T Cells, and CD20+ B cells.

**b. Determine the macrophage population (CD14+) as a percentage of the total immune cell population by flow cytometry (months 1-36).**

We have now analyzed 26 fresh tumors sent from our collaborators at the Brigham & Womens Hospital and from Dr. Jablons by FACS for inflammatory cell populations. Flow studies confirmed and also amplified the results from the immunohistochemical studies (described in Task 1a). Initially we analysed tumors using a 5 color FACS and were thus able to determine the major inflammatory cell populations (**Figure 2**); within the last 4 months, we acquired a high density FACS, able to detect 14 colors and thus to refine the analysis for many inflammatory subtypes (**Figure 3**).

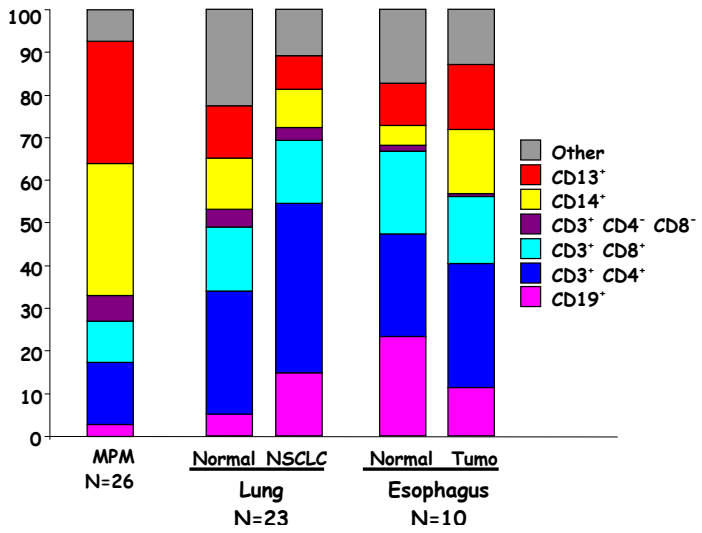


**Figure 2. Mesothelioma tumors studied by 5 color FACS for inflammatory cell populations**. In this study, 26 fresh mesothelioma tumors were disaggregated to single cells, fixed and stained for inflammatory cell surface markers. Mesothelioma tumors are shown to have a high percentage of macrophages (CD14+) and myeloid cells (CD13+) when compared with other thoracic malignancies, lung and esophageal cancer. Mesothelioma appears to have a lower percentage of T (CD4 and CD8) and B cells (CD19) than NSCLC or esophageal CA.

Using high density FACS, we have now studied 5 fresh tumors sent from Boston along with 4 peripheral blood specimens from patients. These high density studies have confirmed the high percentage of macrophages in these tumors (approximately 30% of CD45+ cells). Thus, by immunohistochemistry and flow studies (5 color and 14 color), macrophages represent the predominant inflammatory cell within the inflammatory cell population of mesothelioma tumors.

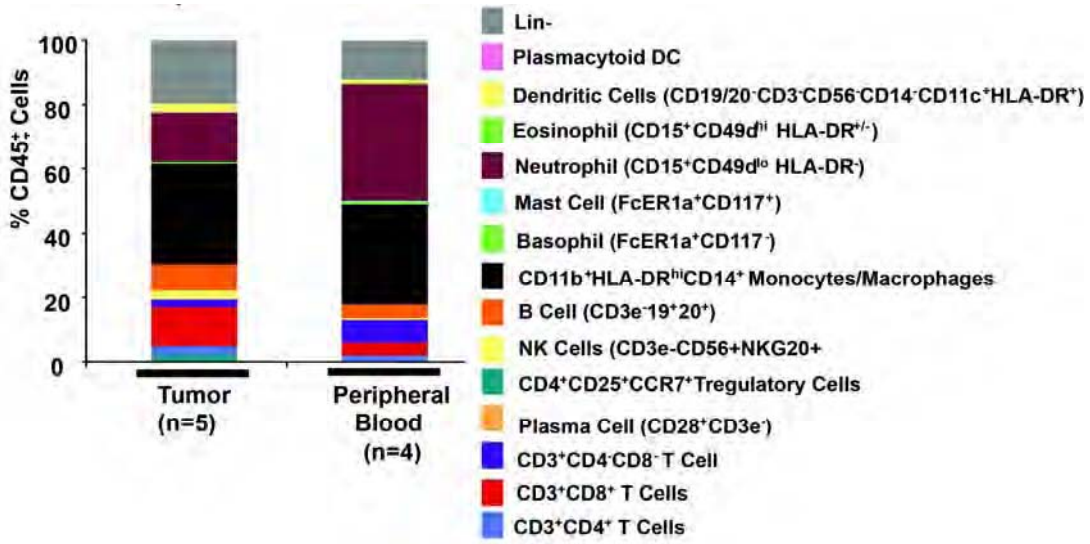


**Figure 3. Tumor and peripheral blood from patients with mesothelioma studied using high density 14 color FACS.** In 5 additional fresh tumors, the findings from high density FACS confirm that macrophages (CD14+) are a high proportion of the inflammatory population. In addition, we could confirm that the myeloid population identified in Figure 3 consisted mainly of neutrophils.

In additional FACS studies, we disaggregated the tumor and stained cells for mesothelin to determine the percentage of cells within tumors that were mesothelioma cells (mesothelin-positive) (**Figure 4**). These results will be of value for future studies in which mesothelin-positive cells will be sorted and used in hybrid spheroid studies. We find that the actual malignant cells constitute approximately 50% of the tumor, which is consistent with the finding that the inflammatory cell population constitutes approximately 30% of the tumor (see **Figure 1A**). The additional 20% of the cell population may consist of non-malignant, non-inflammatory tumor-associated cells such as endothelial cells and fibroblasts.

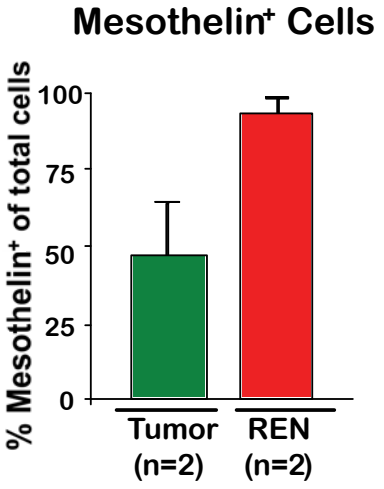


**Figure 4. Mesothelioma cell population in mesothelioma tumors.** In 2 fresh epithelial tumors, we stained the disaggregated cells for mesothelin, a mesothelioma cell surface marker. We found that the mesothelioma cells constitute approximately half of the cells within the tumors. REN mesothelioma cells were used as a positive control.

**c. Determine the profile of other immune cells within the microenvironment of the mesothelioma tumor using a panel of cell surface markers (months 1-36).**

We are continuing to study the profile of the other immune cells using the high density FACS, as described above. This new FACS approach will enable the assessment of activation status within many of the inflammatory cell populations. As an example, we are using the CD69+ activation marker for T cells (CD4 and CD8) to compare the activation status of these T cells in tumor compared to blood from the same patients. In one tumor-blood comparison, the T cells were found to be activated within the tumor (**Figure 5**). The CD69 early activation marker has been reported to be activated in lymphocytes within tumors, leading to proliferation but not necessarily to a cytotoxic effector function. Nonetheless, the presence of T cells with early activation markers indicates the potential for such T cells to be stimulated to a cytotoxic effector function.

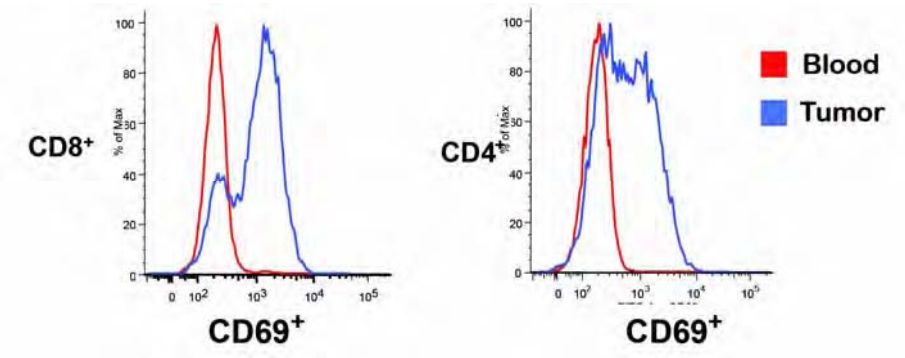


**Figure 5: T cell activation in tumor compared to blood from the same patient.** T cell activation markers such as CD69+ markers indicate that T cells are active in the tumor compared to T cells present in the blood, suggesting that both CD8 and CD4+ T cells are activated within the tumor.

**d. Confirm macrophage percentages by immunodetection of the same mesothelioma in fixed tissues (CD68+) (months 1-36).**

In these ongoing studies, the percentage of macrophages using FACS is being compared to that found by immunohistochemistry. On average, using both immunohistochemistry and flow cytometry, macrophages constitute between 30-70% of the CD45+ (inflammatory) cell population in mesothelioma (**Figures 1B**, **2, 3**). We have also found no signficant difference in macrophage percentage in the same tumor when measured by immunohistochemistry on freshly fixed tissue and when measured 24 h later by flow cytometry.

**e. Isolate macrophages from human mesothelioma disaggregated into single cells by flow cytometry for use in co-culture spheroids with mesothelioma cells (1-12).**

Human macrophages were isolated successfully with high viability (80-90%) using Ficoll separation to remove the non-monocytic cells and then isolating CD14+ cells by bead isolation. These were placed on plastic to adhere and then differentiated using MCSF and 1% FBS for 2 days. Then, the macrophages were polarized with M1 or M2 cytokines (or M0= no cytokines) over 24 h, and they were lightly trypsinized and used to

prepare spheroids with human mesothelioma cell lines. The spheroids were exposed to polarizing cytokines for another 24 h and then treated with TRAIL plus gemcitabine for 24 h. The presence of human macrophages isolated from mesothelioma increased mesothelioma cell apoptotic response to treatment, especially when the macrophages were polarized toward an M1 phenotype (shown in Task 2g below).

**f. Determine macrophage functional properties in mesothelioma using fixed tissues, by tissue microarray, and by immunohistochemistry for protein expression to define their M1 or M2 microenvironmental status (months 1-12)**

Cytokine staining in tissue has proved to be a challenging technical problem and new approaches are being perfected for this and other studies. Fortunately, the fixed tissues are stored and the tumor tissue microarrays generated for these studies so the task is only postponed until he second year of funding. These tumors will serve as a tissue bank for this task and others for the next two years of the project.

**g. Determine the M1 or M2 gene expression signature of macrophages by commercial global chip assay for RNA from tumor tissue or cultures of cells/spheroids for gene profile (months 1-12).**

The quantitative PCR analysis of 7 mesothelioma tumors compared to normal tissues has shown an upregulation of message for several inflammatory cytokines, including CCL3, a key macrophage-produced inflammatory chemokine, and CSF-1, a macrophage stimulating and recruitment factor (**Figure 6**). Interestingly, in these first 7 tissues, we do not yet see a clear polarization signal. The elevation of IL10 is indicative of an M2 phenotype while the increase in IFN gamma would suggest an M1 phenotype. We continue to pursue these studies with additional fresh frozen mesothelioma samples; currently, we have 18 more frozen and ready to analyze. With additional data, including the protein expression data obtained as part of 2f, we expect a clearer picture to emerge. If a clear picture does not emerge, we will plan to disaggregate the mesothelioma cells from the tumor to study them away from the microenvironment. The isolated mesothelioma cells could then be studied for their message for cytokines and studied for their intracellular cytokine concentrations.

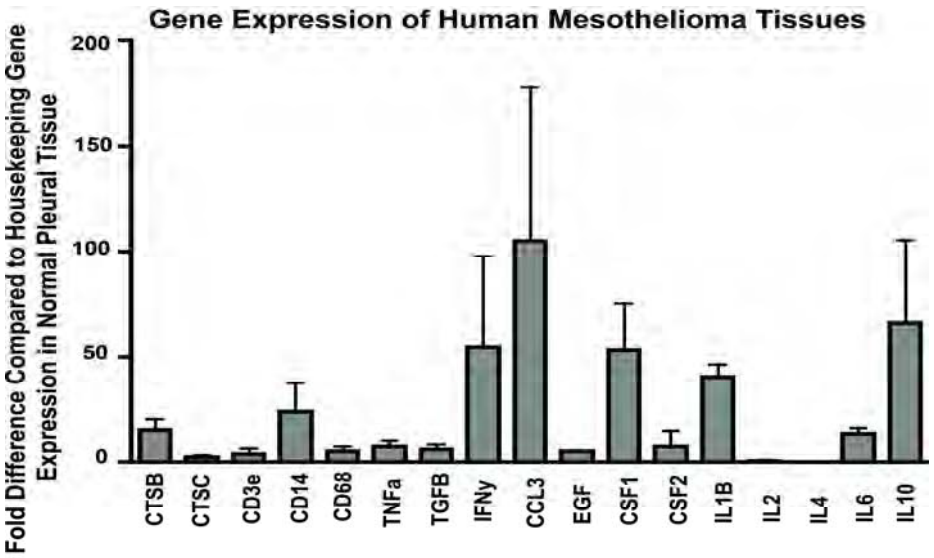


**Figure 6. Quantitative PCR of Human Mesothelioma Tumors**. 7 snap frozen human mesothelioma tumors were analyzed using quantitative PCR to determine gene expression of key genes indicating macrophage polarization. Mesothelioma tumor samples were run alongside normal pleural samples to measure change in gene expression compared to tumor-free patients. There is a significant increase in CD14, IFN-y, IL-10 and CCL3 genes, indicating an inflammatory response. The pattern does not suggest a specific polarization state.

**h. Analyze cytokines produced by mesothelioma tumor fragments by commercial cytokine bead assay from human mesothelioma grown as tumor fragments (months 1-12).**

We have not had success in measuring cytokines from the tumors, despite use of several different assays. We believe the cytokines have been too dilute for consistent results and efforts to concentrate the samples are underway to allow measurement. At the same time, we are taking a different tack, to measure intracellular cytokines via flow cytometry. The protocol is now being perfected in the laboratory and will be applied to mesothelioma tumor tissue. There are several potential advantages of the intracellular cytokine assay compared to an ELISA which measures secreted, soluble cytokines. For one, the dilution of cytokines is not a factor. For another, we can co-stain the cells with an anti-mesothelin or an anti-CD14 antibody to determine what cytokines are produced specifically by the mesothelioma cells or macrophages, respectively. Thus, we have modified our plan for these studies and we expect them to be complete in the next funding cycle.

**i. Analyze cytokines produced by multicellular spheroids made from either mesothelioma cells alone or mesothelioma cells plus macrophages (THP-differentiated) (months 1-12).**

We have completed studies with hybrid spheroids showing that we are able to polarize the mesothelioma + macrophage hybrid spheroids to an M1 or M2 phenotype (**Figure 7**). In addition, we find that the apoptotic treatment using TRAIL or gemcitabine does not

alter the polarization phenotype of the hybrid spheroids. In these studies, we have also begun a more detailed study of M2 polarization, to M2a and M2c, which are induced by exposure to IL-4 and IL-10 respectively.

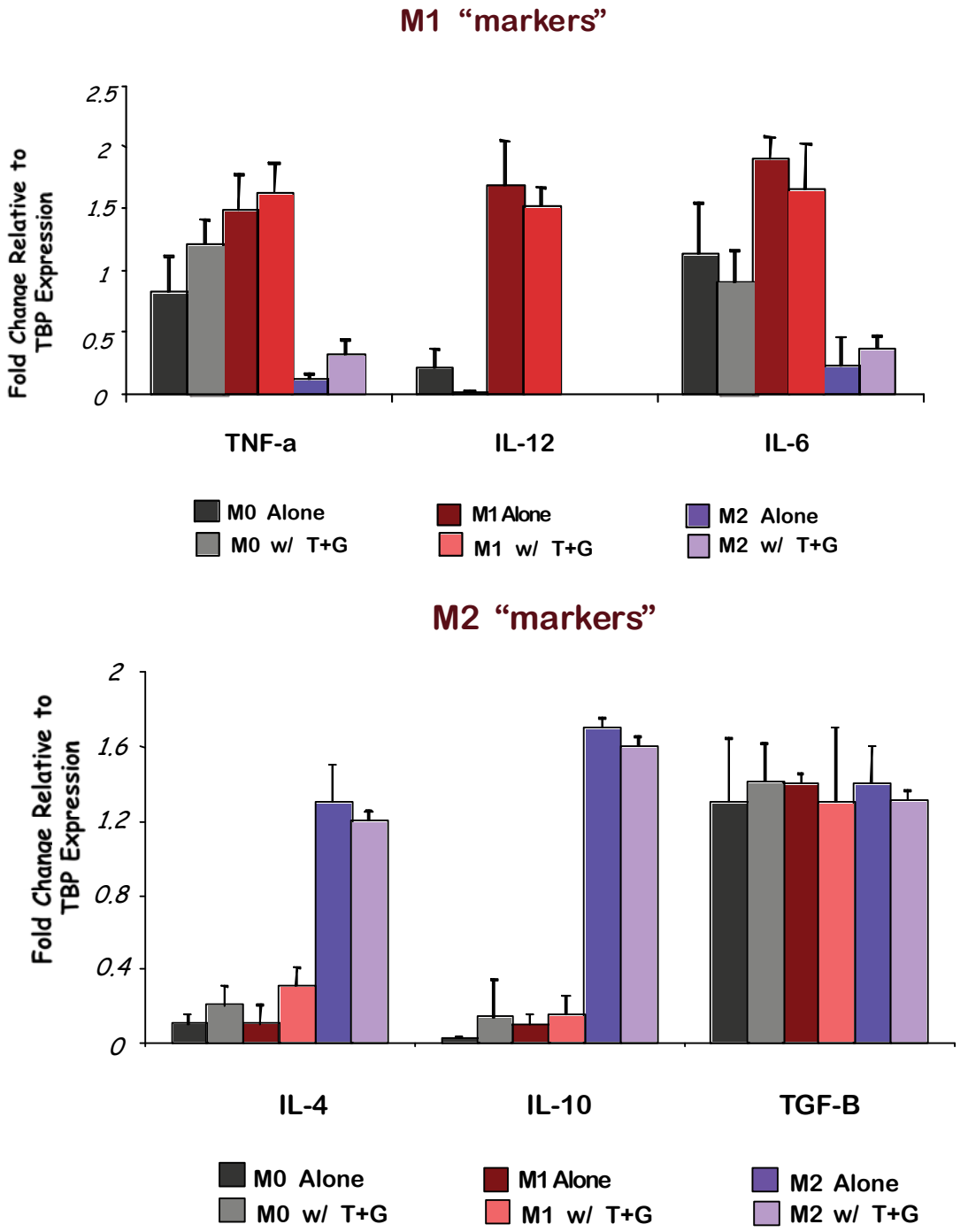


**Figure 7. Cytokine Expression in Hybrid M28 Macrophages is Altered by Programming to an M1 or M2 Phenotype**. Macrophages differentiated from peripheral blood were reprogrammed to either an M1 (IFNy/LPS), M2a (IL4), or M2c (IL10) phenotype over 48 hours in culture. Reprogrammed macrophages were then incorporated with M28 human mesothelioma cells in poly-HEMA coated 96-well plates to form 3D hybrid spheroids. These hybrid spheroids were

grown in DMEM with 1% FBS with any additional polarizing cytokines (M1: IFN-y/LPS, M2a: IL4; M2c: IL-10) for 24 hours. 18-20 spheroids were then harvested and fixed using RLT buffer. Spheroids were then harvested and fixed using RLT buffer. Samples were processed for RNA and cDNA synthesis. Real Time PCR was used to quantify gene expression for M1 Markers: TNF-a, IL-12 p40, and IL-6 as well as M2 markers: IL-4, IL-10, and TGF-B. Expression values were normalized against the expression of housekeeping genes, specifically, TBP.

**j. Analyze changes in cytokine expression when spheroids (mesothelioma only or mesothelioma plus macrophage) are treated with TRAIL or TRAIL plus gemcitabine (months 1-12).**

See above. There was no significant change in the polarization state or cytokine profile after treatment with TRAIL plus gemcitabine (T+G).

***Task 2. To determine the functional significance of macrophages as regulators of mesothelioma apoptosis in vitro.***

**a. Confirm ability of clodronate-liposomes to deplete macrophages from multicellular spheroids made either with THP-1 differentiated macrophage cells or with primary mesothelioma-derived macrophages and determine optimal dose and timing (months 9-14).**

In order to advance the in vivo projects, we used the clodronate more extensively in the in vivo model applying the dosing and timing schedule published for the in vivo setting. The study was readily performed in mice with GFP-labeled macrophages showing that the clodronate did indeed deplete all GFP-labeled cells from the peritoneal lavage in clodronate-treated mice. The studies are now being carried out in hybrid spheroids to establish the optimal dosing for the in vitro setting and should be completed as planned within the next 2 months.

**b. Confirm ability of clodronate-liposomes to deplete macrophages from mesothelioma tumor fragment spheroids with dose and timing established above (months 12-24).**

As above, we will begin these studies as planned this year, following the dose established as effective in the hybrid spheroids above (Task 2a).

**c. Analyze effect of macrophages and of macrophage depletion on apoptosis of mesothelioma cells to treatment with TRAIL or TRAIL plus gemcitabine using multicellular spheroids either with no macrophages, macrophage-depleted or with macrophages (months 12-24).**

We have begun these studies using the no macrophage and with macrophage hybrids, the macrophage-depleted studies will begin this year and will be important to show whether the clodronate itself alters the results. Growing the macrophages with the mesothelioma cells leads to a well formed chimeric structure we call a hybrid spheroid, with the macrophages well distributed throughout the spheroid (**Figure 8A**). We have extensively confirmed our preliminary studies that polarization of the macrophages to an M1 phenotype enhances the apoptosis of mesothelioma cells grown with them (**Figure 8B**). The macrophages that are not polarized (called M0) do not have a significant effect in these in vitro experiments. Polarization of the macrophages to an M1 phenotype however induces a robust pro-apoptotic effect. This effect, as shown in Figure 8 and reproduced in all our experiments, requires the presence of the macrophages; the polarization program has no effect on mesothelioma cells themselves. The pro-apoptotic activity of the macrophages in hybrid spheroids has now been reproduced in more than one cell line (M28 and REN), with at least 4 different sources of macrophages (derived from the THP-monocyte-like cell line, from peripheral blood monocytes, from banked blood monocytes, or from human mesothelioma tissue) and with different apoptotic treatments. We are confident of these findings and have worked to establish the optimal conditions. For example, we have found that freshly obtained macrophages, derived from peripheral blood monocytes, are more effective and active in the ability to induce apoptosis than those derived from banked blood. In certain cases, we have also been able to show that an M2 phenotype suppresses the mesothelioma cell phenotype; this result appears to be more dependent on the culture conditions, requiring a reduction in the background serum stimulation of the cells to 1-2% serum.

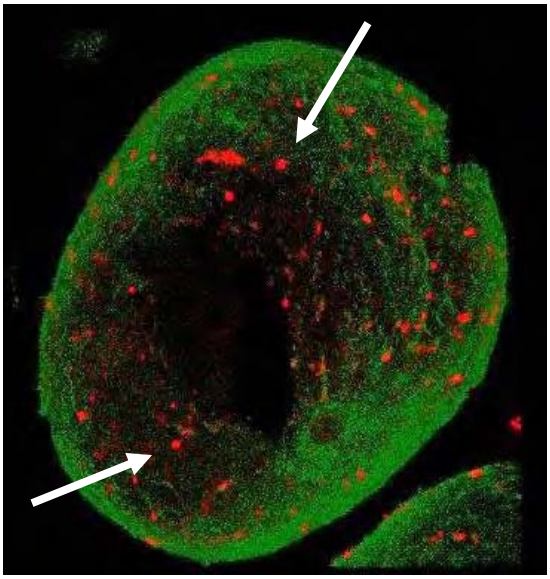

**Figure 8A. Macrophages and mesothelioma cells form hybrid spheroids**. This spheroid contains 10,000 mesothelioma cells (green, anti-cytokeratin) and 500 (5%) macrophages (red, anti-CD69) (see arrows). By fluorescent microscopy, the macrophages are found to be distributed throughout the spheroid. Spheroid is approximately 0.5 mm in diameter.

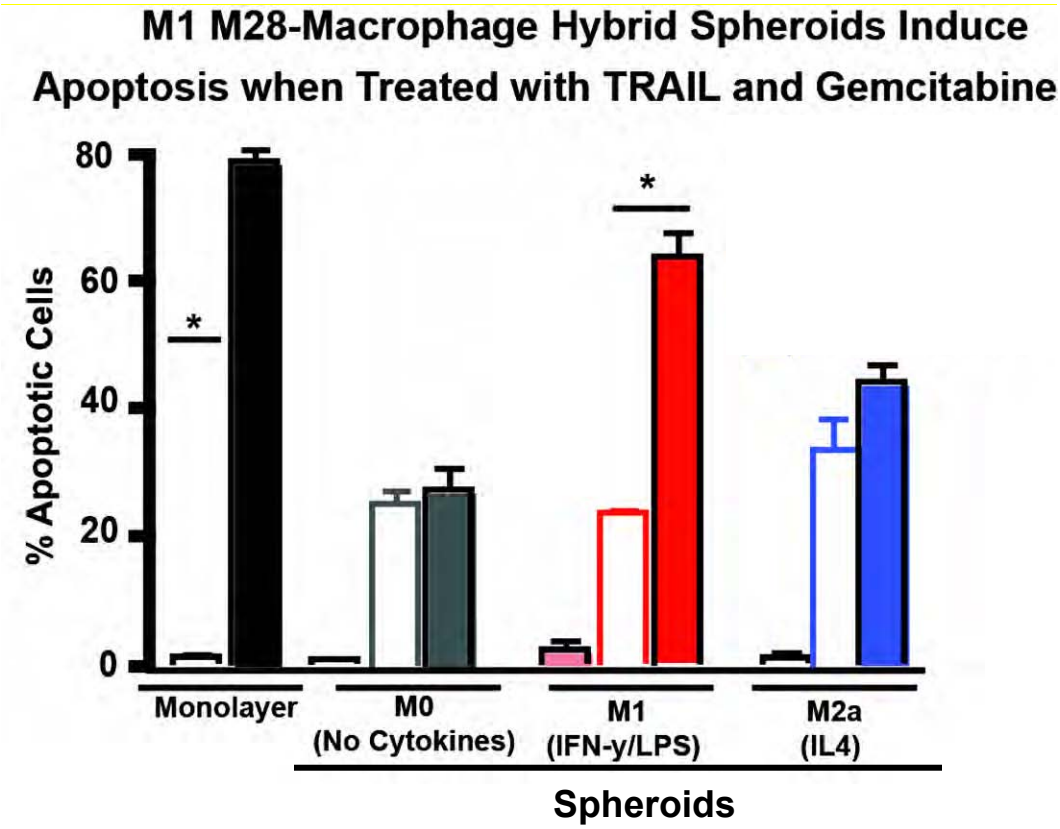


**Figure 8B. Macrophages in mesothelioma spheroids can be polarized to a pro-apoptotic potential.** Hybrid spheroids made with M28 mesothelioma cells and 5% THP-derived macrophages were polarized with cytokines as shown for 24 hours and then exposed to TRAIL plus gemcitabine for 24 hours. Mesothelioma cell apoptosis was increased in hybrid spheroids exposed to M1 cytokines. The degree of apoptosis was nearly equivalent to the apoptosis seen in treated monolayers (black bar), showing that the macrophage effect could significantly undermine the acquired apoptotic resistance of the 3D spheroids. (No macrophages, clear bars; with macrophages, solid gray/red/blue bars; M0, no polarizing cytokines, M1, IFN gamma/LPS; M2a, IL4). The first bars in each group are no TRAIL plus gemcitabine. The second and third bars are after TRAIL plus gemcitabline.

**The Macrophage Pro-Apoptotic Effect is Cytokine-mediated**
The effect of macrophages could be reproduced by use of conditioned media from macrophages polarized by different cytokine environments showing the involvement of soluble mediators. Neutralization of the cytokine tumor necrosis factor (TNF) in the conditioned media reduced the pro-apoptotic effect on the mesothelioma cells (**Figure 9),** suggesting that TNF is an important mediator of the pro-apoptotic effect of macrophages in the hybrid spheroids. A similar experiment using an antibody to IL-10 had no effect (**Figure 10**).

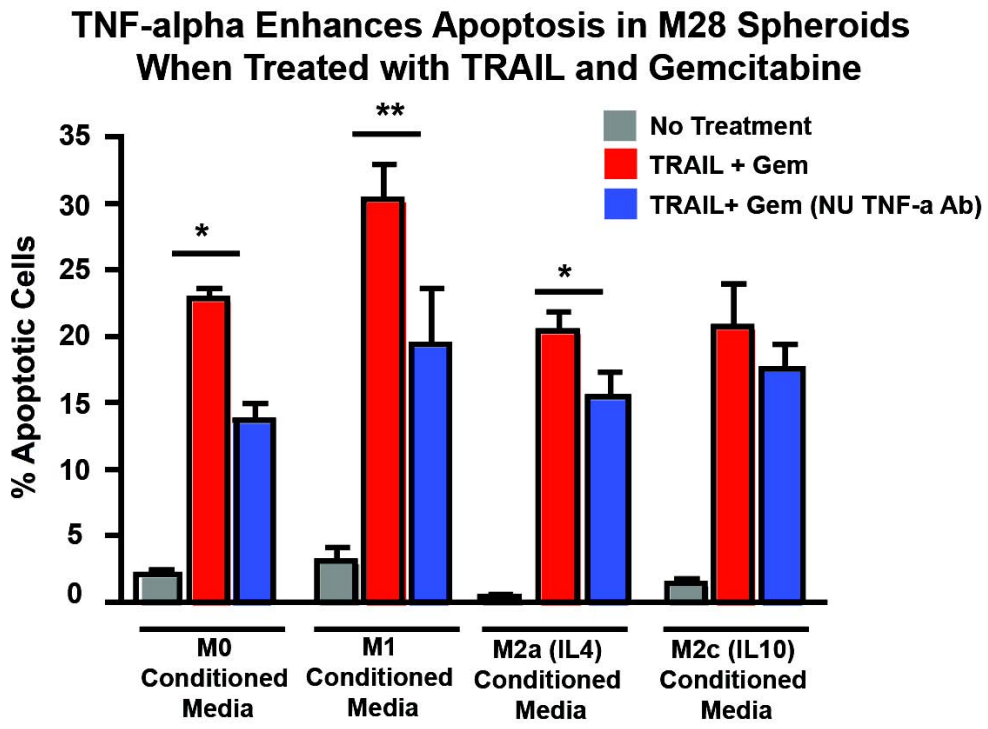


**Figure 9: TNF contributes to the macrophage pro-apoptotic effect on mesothelioma cells.** Monocytes derived from human peripheral blood were exposed to M1 (IFN-y/LPS), M2a (IL-4), and M2c (IL-10) associated cytokines or no cytokines at all for 48 hours in culture. Conditioned media was collected after 48 hours and placed in the upper chamber of a Boyden chamber. 18-20 multicellular spheroids comprised of 10,000 M28 human mesothelioma cells each, were placed in the lower chamber in 600 ul of media containing either no treatment, TRAIL and gemcitabine, or TRAIL and gemcitabine (in the presence of TNF-a neutralizing antibody). Spheroids were incubated for 48 hours and then harvested, disaggregated and fixed using 10% glutaraldehyde in deionized water. Total percentage of apoptotic was quantified by analysis of apoptotic bodies using fluorescent Hoechst staining.

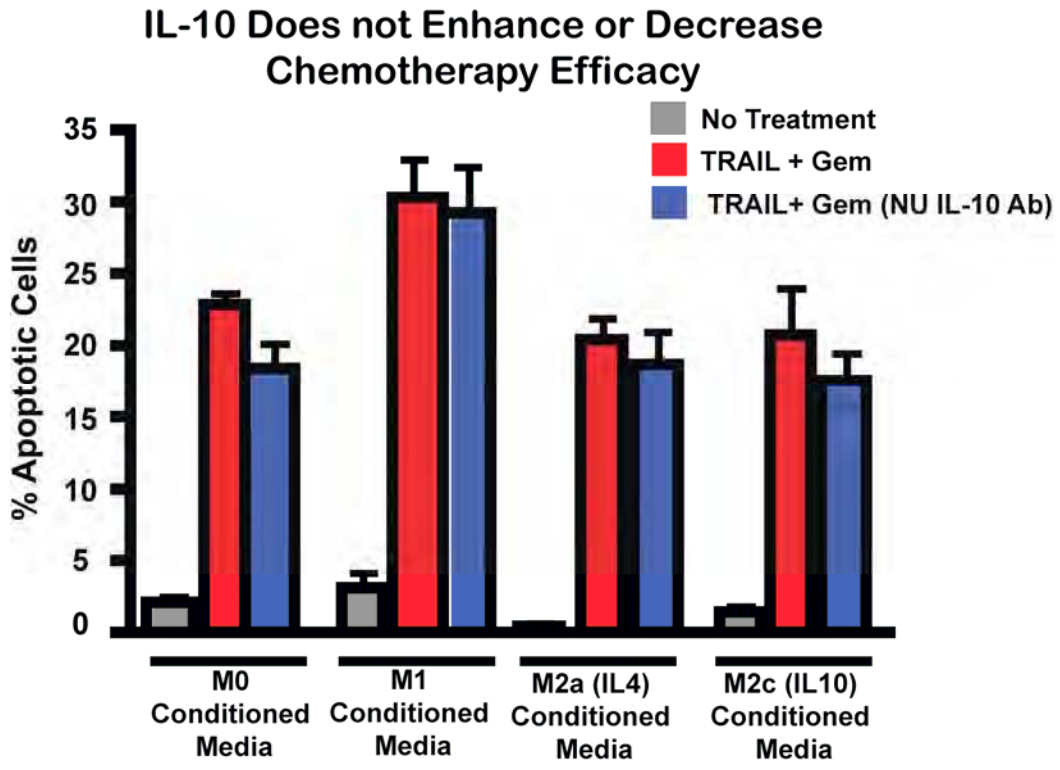


**Figure 10.** Monocytes derived from human peripheral blood were exposed to M1 (IFN-y/LPS), M2a (IL-4), and M2c (IL-10) associated cytokines or no cytokines at all for 48 hours in culture. Conditioned media was collected after 48 hours and placed in the upper chamber of a Boyden chamber. 18-20 multicellular spheroids comprised of 10,000 M28 human mesothelioma cells each, were placed in the lower chamber in 600 ul of media containing either no treatment, TRAIL and gemcitabine, or TRAIL and gemcitabine (in the presence of IL-10 neutralizing antibody). Spheroids were incubated for 48 hours and then harvested, disaggregated and fixed using 10% glutraldehyde in deionized water. Total percentage of apoptotic was quantified by analysis of apoptotic bodies using fluorescent Hoechst staining.

**d. Analyze the effect of macrophages and of macrophage depletion on apoptosis of mesothelioma cells in tumor fragment spheroids to treatment with TRAIL or TRAIL plus gemcitabine (months 12-24).**

These studies are in progress as the conditions continue to be defined in the in vitro hybrid spheroids. These conditions will then be applied to the human tumor fragment spheroids, as planned, within this next funding cycle.

**e. Confirm ability of M1 cytokines to polarize macrophages to a strong M1 phenotype by exposing multicellular spheroids with macrophages (primary mesothelioma or THP-1) to M1 polarizing agents (interferon gamma plus LPS) and confirming with cytokine and gene expression assays for classics M1 or M2 polarization (months 12-24).**

To confirm that we were able to reprogram macrophages, we ran qPCR on our reprogrammed peripheral blood monocytes to evaluate if key M1 and M2 markers were

expressed by the various phenotypes (**Figure 11**). M2 polarized macrophages upregulated M2 markers, such as TGF-B, IL-10, and IL-4; whereas M1 polarized macrophages upregulated markers such as TNFa and IL-12. In these and other experiments, we have not seen a significant difference in the cytokine expression of the different subsets of M2: M2a and M2c.

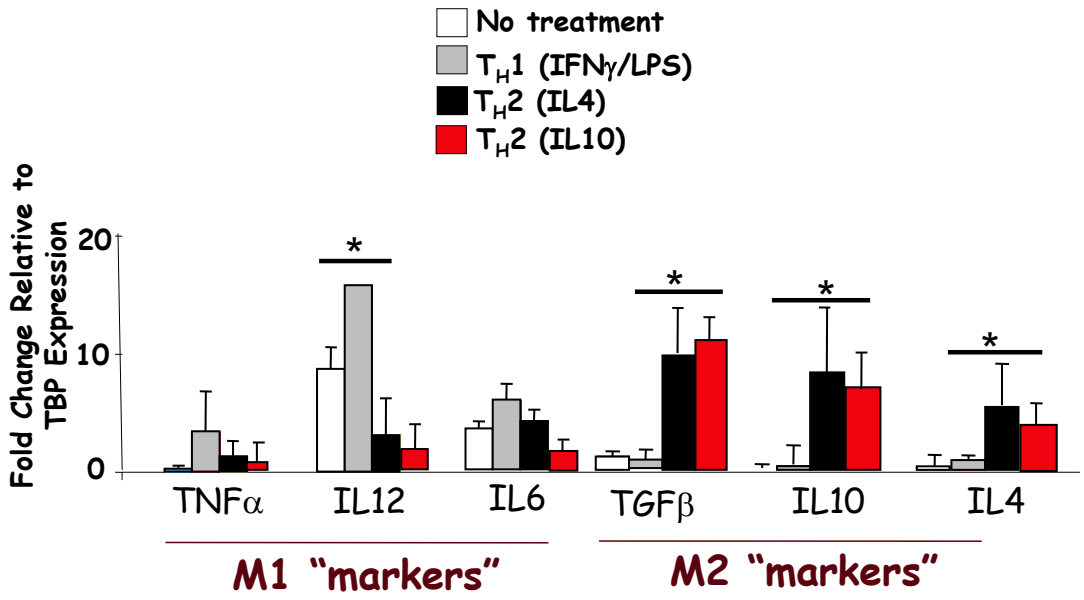


**Figure 11. Human macrophages derived from peripheral blood monocytes can be polarized to an M1 or M2 phenotype.** Peripheral blood monocytes were matured to macrophages and exposed for 24 hours to IFNgamma plus LPS (to stimulate toward a TH1 or M1 phenotype), IL-4 (toward TH2 or M2a) or IL-10 (toward TH2 or M2c). RNA was harvested and analysed by qPCR for expression of message for M1 or M2 marker cytokines.

**f. Polarize macrophages in tumor fragment spheroids using the approach found best above (months 12-24).**

This task is in progress and on track to be completed during the upcoming funding cycle.

**g. Determine whether repolarization of macrophages enhances apoptosis of mesothelioma cells in multicellular spheroids (grown with primary mesothelioma macrophages or THP-1 macrophages) when treated with TRAIL plus gemcitabine (months 12-24).**

See **Task 2c** above showing that repolarization of THP-derived macrophages towad an M1 phenotype enhances apoptosis in MCS. In addition, experiments were performed using macrophages isolated from human mesothelioma tumor tissue (**Figure 12**). Human macrophages isolated from tumor tissue thus retain the ability to enhance apoptosis when polarized to an M1 phenotype. Such results support the feasibility of repolarizing human macrophages in vivo to an M1 and more pro-apoptotic phenotype. Human mesothelioma macrophages show the plasticity of phenotype that can potentially be harnessed to a therapeutic advantage.

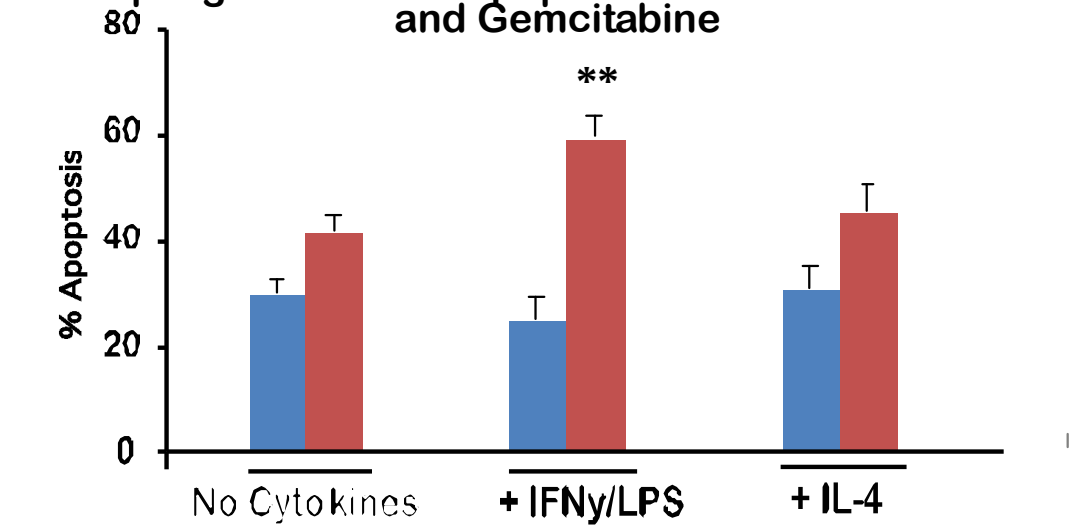


**Figure 12. M1 programmed hybrid M28-Human mesothelioma macrophages enhance apoptosis when treated with TRAIL and Gemcitabine.** 18-20 spheroids each comprised of 10,000 M28 human mesothelioma cells alone (*blue bars*) were treated with polarizing cytokines of either (a) M0 – no cytokines (b) M1 – IFN-y/LPS or (c) M2a – IL-4. In parallel to these spheroids, hybrid spheroids (*red bars*) comprised of 9,500 M28 mesothelioma cells and 500 macrophages isolated and differentiated from human malignant pleural mesothelioma tumors were treated with polarizing cytokines of either (a) M0 – no cytokines (b) M1 – IFN-y/LPS or (c) M2a – IL-4 for 24 hours. These hybrid spheroids were then treated with TRAIL and gemcitabine for 24 hours in addition to their respective polarizing cytokines. Hybrid and M28 spheroids were then harvested, fixed and analyzed for apoptosis using Hoescht staining to quantify the total % of apoptotic cells. *(Blue, no macrophages; red, with macrophages) (**, $p<0.05$ significant increase in apoptosis in hybrid spheroids polarized to M1 compared to M0 or M2 phenotype)*

**h. Determine whether repolarization of macrophages enhances apoptosis of mesothelioma cells in tumor fragment spheroids (months 12-24).**

In progress.

***Task 3. Define functional significance of macrophage depletion or repolarization on mesothelioma survival in vivo.***

**a. Characterize immune cell profile of the murine mesothelioma model induced by intraperitoneal asbestos injections in the NF2+/- mouse model (months 0-24).**

We have begun the mesothelioma model in which NF2+/- mice, with a genetic predisposition for the development of asbestos-induced mesothelioma, are injected every 3 weeks with crocidolite asbestos and followed many months for the development of mesothelioma, as reported. We currently have a cohort of 20 mice that have completed the 8 injections and we have an additional cohort of 30 mice that are in the process of receiving the 8 asbestos injections.

**b. Deplete in vivo macrophages with intraperitoneal clodronate-liposomes in mice without mesothelioma to establish protocol (months 4-12).**

We have followed the in vivo protocol for the use of intraperitoneal clodronate for macrophage depletion. In GFP-macrophage mice, the clodronate was able to eliminate the GFP signal and all cells costaining with two macrophage markers, CD11b+ and F4/80. Thus, the clodronate is effective in removing macrophages when introduced on the schedule as reported. In the first in vivo mesothelioma study (see Task 3d below), clodronate over 3 injections (day 0, 5, 10), led to a depletion of macrophages in the peritoneal lavage by flow cytometric analysis of macrophage markers. Further studies in mice with mesothelioma and in vitro tumor fragment spheroids and multicellular spheroids will be needed to confirm that this regimen of clodronate also removes macrophages from tumor tissues.

**c. Deplete in vivo macrophages in mice with mesothelioma to confirm depletion, to establish effect on other immune cell populations, and to confirm lack of toxicity on the mice (months 12-24).**

In the 40L mesothelioma mice, the tumor cells were injected at day 0 and clodronate was started at day 21 and continued every 5 days until day 40. A FACS analysis at day 40 before treatment began with TRAIL plus gemcitabine showed a significant reduction in macrophages. From this in vivo experiment (see Task 3d below), we have ascites fluid stored and fixed in preparation for flow studies and tissue fixed for immunohistochemistry that will provide information on the effect of clodronate and of TRAIL plus gemcitabine on immune cell populations. In our first experiments with clodronate, there was no evidence of toxicity. In fact, the clodronate-treated mice ultimately did better than the other mice with fewer tumors and appeared healthier. Our next in vivo studies will utilize blood creatinine measurements, weekly weights and other metrics to monitor for any toxicity of the clodronate.

**d. Determine whether macrophage depletion in mice with mesothelioma enhances apoptotic and therapeutic response of mice with mesothelioma to TRAIL plus gemcitabine over 1-4 weeks after treatment (months 24-36).**

Because the NF2 +/- asbestos mesothelioma model takes many months to develop, we have begun a second model of mesothelioma in immunocompetent mice produced by the IP injection of a syngeneic murine mesothelioma line (40L) in C57/Bl/6 mice. In this model, intraperitoneal mesothelioma forms 3-4 weeks after the ip injection of $2 \times 10^6$ 40L

cells. This model was developed by Dr. Agnes Kane, who kindly provided this cell line and a second line (7) for these studies [9]. These more rapid and reproducible models will enable us to study normal inflammatory cell recruitment and manipulation and to refine our treatment dosing and imaging in preparation for the more challenging, expensive and time-consuming but ultimately more relevant asbestos-induced NF+/- mouse model. The first studies in the 40L peritoneal mesothelioma model have already yielded exciting results.

We have performed one experiment in mice with the 40L peritoneal mesothelioma, using clodronate-liposomes and then TRAIL plus gemcitabine to learn whether the clodronate-mediated depletion of macrophages will sensitize the tumor cells to chemotherapy. We have found that the mice treated with clodronate had smaller tumors at 20 days, as had been reported by Dr. Kane's group [9]. However, we have extended these studies to show that the depletion of the macrophages sensitizes the tumor to chemotherapy, leading to mice with smaller, fewer tumors or in some cases no visible tumor at all (**Figure 13 and 14**).

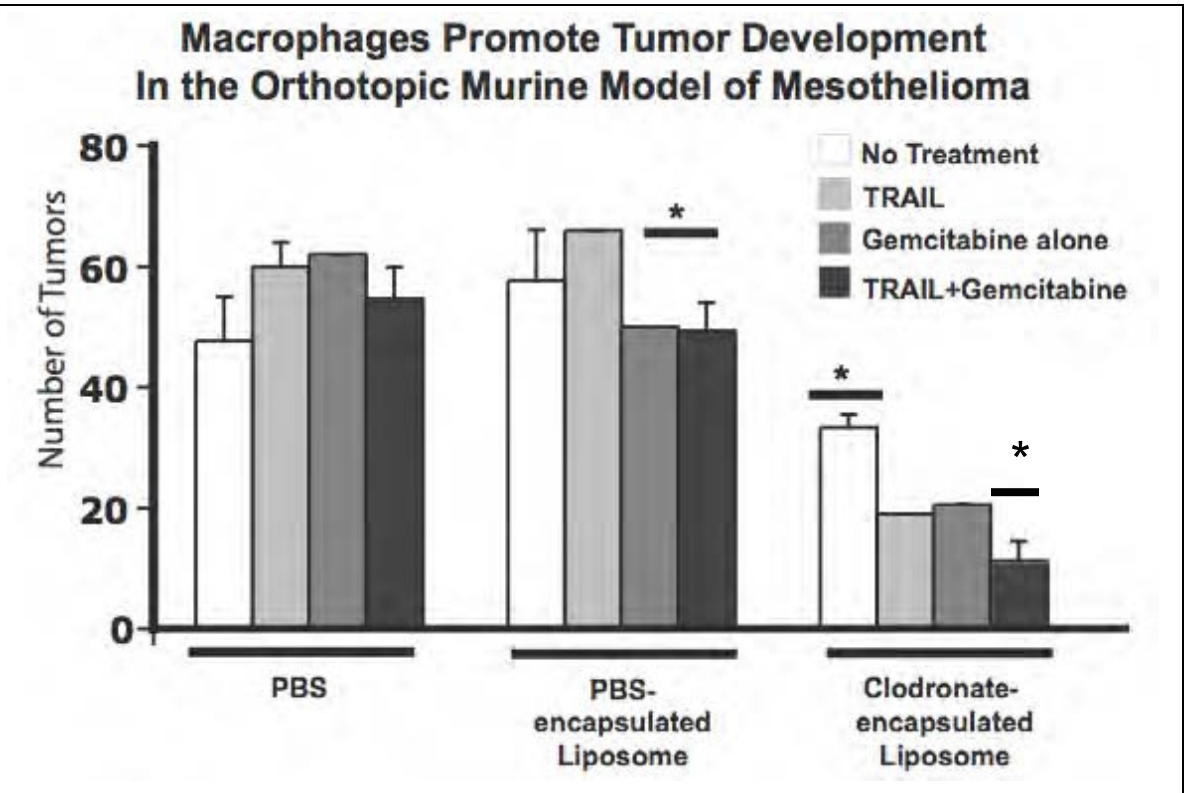


**Figure 13. Clodronate increases the sensitivity of mesothelioma to TRAIL plus gemcitabine treatment.** In 39 mice (3-4 in each condition), 2 x $10^6$ syngeneic murine mesothelioma 40L cells were injected to produce an ip mesothelioma. After 21 days, when tumors start to be apparent, clodronate-liposomes, PBS-liposomes or PBS alone (100 uL) was injected ip every 5 days for 20 days. At this point, the mice were treated with TRAIL alone, gemcitabine alone or the combination of TRAIL plus gemcitabine ip for 10 days. The experiment was terminated when mice in the PBS and PBS-liposome group were losing weight and appearing listless. At this point (50 days after injection of tumor cells), the number of tumors in the mesentery and peritoneum and diaphragm is shown.

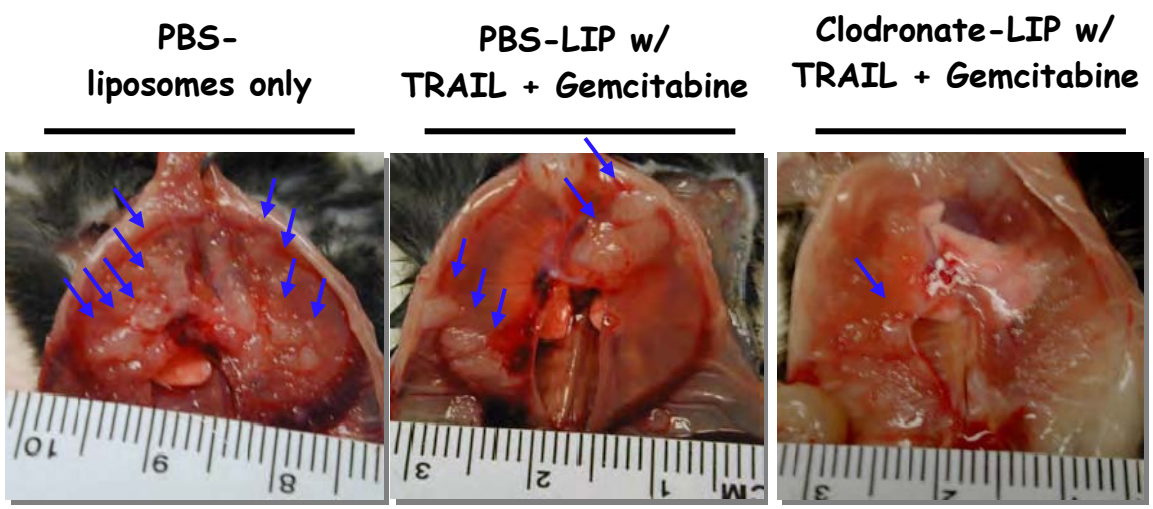


**Figure 14. Representative photographs of the inferior surface of the diaphragm of mice after TRAIL plus gemcitabine.** In the PBS-treated mouse to the left, multiple tumors lie in a confluent pattern across the diaphragm (see multiple blue arrows). In the center photo (PBS-treated and TRAIL plus gemcitabine), there are approximately 8-10 tumors seen (see blue arrows). In the rightmost photo, from a mouse treated with clodronate followed by TRAIL plus gemcitabine, only one tumor is seen (blue arrow).

These studies have led to several plans for further experiments. The 40L cells are now being transfected with a luciferase construct so that, in the next experiments, the tumor size can be monitored by luciferase optical imaging. This will also be done with the second cell line, the 7 cells, so that additional experiments with a second cell line can be used to confirm these findings.

Cells, spheroids and tissues have been collected to be analyzed by FACS for immune cell populations, RNA for cytokine expression, immunohistochemistry for macrophage numbers and for apoptosis (cleaved caspase 3) as well as for confirmation of tumor morphology and burden.

**e. Repolarize macrophages in vivo by injections of interferon gamma plus LPS and assaying cytokines of peritoneal ascites and gene expression of peritoneal macrophages obtained by lavage to confirm method of repolarization, and to select best tolerated method for the mice (months 12-24).**

We are on target to accomplish this task by the third funding cycle.

**f. Determine whether repolarization of macrophages in vivo enhances the apoptotic and therapeutic response of mice with mesothelioma to TRAIL plus gemcitabine over 1-4 weeks after treatment (months 24-36).**

We are on target to accomplish this task in the third funding cycle.

**g. Determine whether the depletion or repolarization of macrophages enhances survival of mice with mesothelioma after treatment with vehicle, TRAIL or TRAIL plus gemcitabine (months 24-36).**

We are on target to accomplish this task in the third funding cycle.

## III. KEY RESEARCH ACCOMPLISHMENTS

***Task 1. To determine the functional significance of macrophage phenotype on mesothelioma cell survival.***

a. Elucidate the percentage of immune cells (CD45+) in human mesothelioma tumors and correlate immune cell infiltration with histopathologic subtype (months 1-6).

- Acquired 71 fixed and paraffin-embedded mesothelioma tumor samples
- Prepared mesothelioma tumor tissue microarrays for immunohistochemistry
- Determined the pathologic subtype of each tumor in concert with the thoracic pathologist, Dr. Steven Nishimura
- Quantified each sub-population as a percentage of the total inflammatory cell population using digital imaging software, the Aperio system, optimized for either membrane or nuclear staining
- Correlated presence of inflammatory cell populations with mesothelioma pathologic subtype
- Established collaboration with a pulmonary laboratory of Dr. Michael Matthay to obtain normal human pleura

b. Determine the macrophage population (CD14+) as a percentage of the total immune cell population by flow cytometry (months 1-36).

- Optimized disaggregation protocol for mesothelioma tumors
- Analysed the CD14+ macrophage population in 26 fresh tumors
- Established that the percentage of CD14+ cells in the mesothelioma inflammatory population exceeds that of the other thoracic tumors, lung and esophagus
- Expanded flow cytometry studies to a new high density 14 color system
- Confirmed the high percentage of macrophages using high density flow
- Analyzed 26 fresh tumors using 5 color flow cytometry
- Analyzed 5 tumors so far using 14 color, high density flow cytometry
- Initiated collection of peripheral blood samples from the patients with mesothelioma to compare activation status of cells in tumor compared to periphery
- Included mesothelin in panel of cell surface markers to quantify percentages of mesothelioma cells in tumors

c. Determine the profile of other immune cells within the microenvironment of the mesothelioma tumor using a panel of cell surface markers (months 1-36).

- Performed immunohistochemical analysis on 71 tumors to identify inflammatory cell infiltrates, e.g. CD45, CD68, CD8, CD4 and CD20

d. Confirm macrophage percentages by immunodetection of the same mesothelioma in fixed tissues (CD68+) (months 1-36).

- Confirmed the percentages of macrophages in mesothelioma using same tumors studied in immunohistochemistry (by CD68+) and in flow cytometry (by CD14+)

e. Isolate macrophages from human mesothelioma disaggregated into single cells by flow cytometry for use in co-culture spheroids with mesothelioma cells (1-12).

- Isolated macrophages from fresh mesothelioma tumors with high viability (80-90%) and in sufficient numbers to study
- Produced hybrid spheroids with macrophages isolated from human mesothelioma tumors

f. Determine macrophage functional properties in mesothelioma using fixed tissues, by tissue microarray, and by immunohistochemistry for protein expression to define their M1 or M2 microenvironmental status (months 1-12)

- Stored mesothelioma tissues in tissue bank for these studies while technique is being tested in laboratory

g. Determine the M1 or M2 gene expression signature of macrophages by commercial global chip assay for RNA from tumor tissue or cultures of cells/spheroids for gene profile (months 1-12).

- Quantified cytokine message in 7 snap frozen mesothelioma tumors
- Compared message to 4 normal pleural samples

h. Analyze cytokines produced by mesothelioma tumor fragments by commercial cytokine bead assay from human mesothelioma grown as tumor fragments (months 1-12).

- Determined that cytokines were secreted by tumor fragment spheroids, but were too dilute for accuracy
- Initiated testing of an intracellular cytokine assay
- Developed mesothelin staining and gating by flow cytometry for use in this intracellular and other flow cytometry studies to identify mesothelioma cell population

i. Analyze cytokines produced by multicellular spheroids made from either mesothelioma cells alone or mesothelioma cells plus macrophages (THP-differentiated) (months 1-12).

- Quantified expression of key M1 or M2 cytokines in multicellular spheroids e.g M1 (TNF, IL-12, IL-6) or M2 (IL-4, IL-10, TGF) compared to housekeeping gene TBP
- Determined that the polarization protocol does polarize hybrid spheroids to an M1 or M2 phenotype

j. Analyze changes in cytokine expression when spheroids (mesothelioma only or mesothelioma plus macrophage) are treated with TRAIL or TRAIL plus gemcitabine (months 1-12).

- Determined that treatment with TRAIL or TRAIL plus gemcitabine has no significant effect on the M1 or M2 phenotype

***Task 2. To determine the functional significance of macrophages as regulators of mesothelioma apoptosis in vitro.***

a. Confirm ability of clodronate-liposomes to deplete macrophages from multicellular spheroids made either with THP-1 differentiated macrophage cells or with primary mesothelioma-derived macrophages and determine optimal dose and timing (months 9-14).

- Obtained and prepared clodronate-liposomes for in vivo use
- Use in vitro systems not initiated in months 1-12

b. Confirm ability of clodronate-liposomes to deplete macrophages from mesothelioma tumor fragment spheroids with dose and timing established above (months 12-24).

- Obtained and prepared clodronate-liposomes for in vivo use
- Use in vitro systems not initiated in months 1-12

c. Analyze effect of macrophages and of macrophage depletion on apoptosis of mesothelioma cells to treatment with TRAIL or TRAIL plus gemcitabine using multicellular spheroids either with no macrophages, macrophage-depleted or with macrophages (months 12-18).

- Derived macrophages from peripheral blood and THP monocyte-like cells
- Produced hybrid spheroids with two different mesothelioma cell lines (REN, M28) combined with macrophages derived from 4 different sources (THP, peripheral blood, banked blood, mesothelioma tumors)
- Established an imaging protocol to show that macrophages were viable and well distributed within spheroids
- Determined that macrophages alone (without polarization) had no consistent effect on the mesothelioma cell apoptotic response to treatment

d. Analyze the effect of macrophages and of macrophage depletion on apoptosis of mesothelioma cells in tumor fragment spheroids to treatment with TRAIL or TRAIL plus gemcitabine.

- Not initiated in months 1-12. Plan to use optimal protocol worked out in Task 2c in multicellular, hybrid spheroids

e. Confirm ability of M1 cytokines to polarize macrophages to a strong M1 phenotype by exposing multicellular spheroids with macrophages (primary mesothelioma or THP-1) to M1 polarizing agents (interferon gamma plus LPS) and confirming with cytokine and gene expression assays for classics M1 or M2 polarization (months 12-24).

- Determined that M1 stimulation (IFN gamma plus LPS) does polarize macrophages to an M1 phenotype (e.g. expressing TNF, IL12 and IL6)
- Determined that M2 stimulation (IL4 - M2a or IL10 - M2c) does polarize macrophages to an M2 phenotype (e.g. expressing TGF beta, IL10 and IL4)

f. Polarize macrophages in tumor fragment spheroids using the approach found best above (months 12-24).

- Not initiated in months 1-12.

g. Determine whether repolarization of macrophages enhances apoptosis of mesothelioma cells in multicellular spheroids (grown with primary mesothelioma macrophages or THP-1 macrophages) when treated with TRAIL plus gemcitabine (months 12-24).

- Determined that the presence of M1 polarized macrophages consistently enhances mesothelioma cell apoptotic responses to TRAIL plus gemcitabine
- Showed that this conclusion does not depend on the source of macrophages although peripheral blood monocyte and THP-derived macrophages appear most robust
- Showed, in expanded studies, that this pro-apoptotic effect was mediated by soluble factors and could be reproduced by exposing the mesothelioma cells to media conditioned by M1 polarized macrophages
- Determined that the cytokine TNF (an M1 cytokine), but not IL-10 (an M2 cytokine), contributed to the macrophage pro-apoptotic effect

h. Determine whether repolarization of macrophages enhances apoptosis of mesothelioma cells in tumor fragment spheroids (months 12-24).

- Not initiated in months 1-12

***Task 3. Define functional significance of macrophage depletion or repolarization on mesothelioma survival in vivo.***

a. Characterize immune cell profile of the murine mesothelioma model induced by intraperitoneal asbestos injections in the NF2+/- mouse model (months 0-24).

- Established an NF2+/- mouse cohort of 20 mice that have completed the required 8 every 3 weeks intraperitoneal asbestos injections for production of mesothelioma
- Initiated a further cohort of 30 mice that are in process of receiving the 8 asbestos ip injections
- Established a second immunocompetent mouse model of mesothelioma using the 40L murine mesothelioma cell line obtained from Dr. Agnes Kane
- Carried out one experiment in the 40L mesothelioma model showing that mesothelioma develops in 4 weeks, as reported by Dr. Kane
- Characterization of immune cell population in these two models (asbestos induced NF2+/- and syngeneic orthotopic 40L) are planned for months 12-36

b. Deplete in vivo macrophages with intraperitoneal clodronate-liposomes in mice without mesothelioma to establish protocol (months 4-12).

- Depleted intraperitoneal macrophages successfully using clodronate-embedded liposomes from GFP-macrophage labeled mice by showing loss of GFP signal and loss of cells co-staining with F4/80 and CD11b+

c. Deplete in vivo macrophages in mice with mesothelioma to confirm depletion, to establish effect on other immune cell populations, and to confirm lack of toxicity on the mice (months 12-24).

- Used clodronate-liposomes in mice with 40L orthotopic mesothelioma
- Collected tissues after clodronate treatment to determine whether clodronate used in this schedule depleted all tumor-associated macrophages

d. Determine whether macrophage depletion in mice with mesothelioma enhances apoptotic and therapeutic response of mice with mesothelioma to TRAIL plus gemcitabine over 1-4 weeks after treatment (months 24-36).

- Carried out one experiment in which mice implanted ip with 40L syngeneic mesothelioma cells were first given clodronate-liposomes (or PBS or PBS-liposomes) intraperitoneally and then treated with nothing, TRAIL alone, gemcitabine alone or TRAIL plus gemcitabine
- Showed that clodronate-treatment itself was associated with a lower tumor burden
- Found that clodronate-treatment increased the efficacy of the treatment with TRAIL plus gemcitabine
- Collected ascites and tissues for determining presence of macrophages, other immune cells, tumor burden and apoptotic cells

e. Repolarize macrophages in vivo by injections of interferon gamma plus LPS and assaying cytokines of peritoneal ascites and gene expression of peritoneal macrophages obtained by lavage to confirm method of repolarization, and to select best tolerated method for the mice (months 12-24).

- Not initiated in months 1-12

f. Determine whether repolarization of macrophages in vivo enhances the apoptotic and therapeutic response of mice with mesothelioma to TRAIL plus gemcitabine over 1-4 weeks after treatment (months 24-36).
- Not initiated in months 1-12

g. Determine whether the depletion or repolarization of macrophages enhances survival of mice with mesothelioma after treatment with vehicle, TRAIL or TRAIL plus gemcitabine (months 24-36).
- Not initiated in months 1-12

## IV. REPORTABLE OUTCOMES

### A. MANUSCRIPTS

Months 1-12 (Provided as Appendix Material in this Progress Report)

- DeNardo DG, Andreu P, **Coussens LM**. (2010) Interactions between lymphocytes and myeloid cells regulate pro- versus anti-tumor immunity. *Cancer Metastasis Rev, 29(2):309-316*, PMID: 20405169

### B. ABSTRACTS

Months 1-12 (Provided as Appendix Material in this Progress Report)

### C. PRESENTATIONS

**Symposia and Workshops: International**

**Symposia and Workshops: National**

2009 Broaddus, V. Courtney. *Role of macrophages in apoptotic resistance of mesothelioma.* In Workshop on Preclinical Drug and Target Discovery Pipeline. International Mesothelioma Program, Brigham and Womens Hospital, Harvard Medical School, Boston, MA, USA.

2010 Coussens, Lisa M. PLENARY LECTURE. *Regulation of protumor immunity and cancer development.* 2010 *Annual Meeting of the American Association for Cancer Research*, Washington DC USA.

2010 Jablons, David M. *Exploiting emerging biology for the treatment of malignant mesothelioma.* In Symposium entitled: Translational Initiatives in Mesothelioma. American Association of Cancer Research, Washington DC, USA.

2010 Coussens, Lisa M. *Regulation of protumor immunity and cancer development.* IN: *3rd Annual Wyeth Discovery Frontiers in Human Disease Symposium*, New York, NY USA

**Invited Lectures/Seminars: International**

**Invited Lectures/Seminars: National**

2010 Coussens, Lisa M. *Inflammation and cancer: polarized immune responses regulate cancer development.* Cold Spring Harbor Laboratory, CSH NY USA

2010 Broaddus, V. Courtney. *Current studies under the DOD grant mechanism: macrophages and their contribution to mesothelioma.* Mesothelioma Applied Research Foundation, Washington, DC, USA

**Presentations by Coussens or Broaddus Lab Members:**
Nikita Kolhatkar (Coussens and Broaddus' lab, pre-doctoral student)

2009 Kohatkar, Nikita. *Macrophages contribute to mesothelioma chemoresistance.* Poster presentation. Annual UCSF Pulmonary Research Retreat, San Francisco, CA.

2010 Kohatkar, Nikita. Targeting macrophages as a novel therapeutic approach for malignant pleural mesothelioma. Invited presentation. American Association for Cancer Research. Washington, DC.

**D. PATENTS AND LICENSES:** None

**E. DEGREES OBTAINED:** None

**F. REAGENT DEVELOPMENT:**
• Preparation of tumor tissue microarrays prepared with 71 mesothelioma tumors
• Preparation of tumor tissue microarrays stained for CD68, CD4, CD8 and a multitude of inflammatory markers
• Generation of hybrid multicellular spheroids with macrophages derived from THP cell lines
• Generation of hybrid multicellular spheroids with macrophages derived from peripheral blood monocytes
• Generation of hybrid multicellular spheroids with macrophages derived from macrophages isolated from fresh human mesothelioma
• Collection of peripheral blood from patients with mesothelioma
• Collection of frozen mesothelioma tissue from patients at the time of surgery to be used for RNA extraction and qPCR

**G. FUNDING APPLIED FOR BASED ON WORK SUPPORTED BY THIS FUNDING:**

• Barbone, Dario. Mesothelioma Applied Research Foundation. Pending
• Barbone, Dario. SPORE mechanism Career Development Award. Pending.

**H. EMPLOYMENT/RESEARCH OPPORTUNITIES APPLIED FOR:**

• Kolhatkar, Nikita. Accepted to a postdoctoral research program in immunology at The University of Washington, Seattle, WA.

## V. CONCLUSION

Inflammation is now a recognized as promoting solid tumor growth and survival. The inflammatory cells release cytokines and soluble mediators that *directly* promote growth and survival of the malignant cells, and *indirectly* support the tumor by inducing angiogenesis or by suppressing effective cytotoxic T-cell functions. Each tumor now appears to have a unique inflammatory cell milieu. From our studies to date, mesothelioma appears to have an innate inflammatory profile, with a predominance of macrophages and neutrophils, whereas the other thoracic malignancies studied have a more adaptive profile.

The macrophage is the major inflammatory cell type in mesothelioma and, as such, may play a powerful role in mesothelioma development, maintenance and resistance to chemotherapy. Our studies to date have not yet shown the phenotype of these tumor-associated macrophages within their complex microenvironment although we have been able to isolate the macrophages to demonstrate that they retain plasticity and can be polarized to an M1 or an M2 phenotype. We have also completed studies showing that macrophages, when polarized to an M1 phenotype, alter the sensitivity of the mesothelioma cells to therapy. This sensitization to chemotherapy does not require contact but can be reproduced by conditioned media from the macrophages.

Our most exciting preliminary data comes from the first study in immunocompetent mice with orthotopic syngeneic mesothelioma cells. Using clodronate to deplete macrophages, we found that the tumors that formed were smaller (as reported by Dr. Kane's group) but were also more sensitive to subsequent chemotherapy. This preliminary study shows the exciting potential for manipulation of the macrophage in the tumor environment. If depletion of the macrophages can produce a significant improvement in mesothelioma treatment in this one model, then polarization to an M1 phenotype may have even a greater benefit.

In our second funding cycle, we will aim to answer more of the intriguing questions about the role of the macrophage in mesothelioma and about our ability to block it or manipulate it to improve chemoresponsiveness. We will focus on the in vitro hybrid and ex vivo tumor fragment models to identify the molecular mechanisms of the macrophage-mesothelioma interaction and on our two in vivo models of mesothelioma (40L syngeneic orthotopic and NF2+/- -asbestos-induced) to alter macrophage function. The macrophage, the predominant inflammatory cell in all the subtypes of mesothelioma, is already infiltrated throughout the tumor mass. Manipulation of this powerful cell type has the potential to transform mesothelioma from a chemoresistant, intractable and incurable tumor to one more sensitive to current or novel therapies.

## VI. BIBLIOGRAPHY

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4. Pollard, J.W., *Macrophages define the ivasive microenvironment in breast cancer.* J. Leukocyte Biol., 2008. **84**: p. 1-8.
5. Dave, S.S., et al., *Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells.* N. Engl. J. Med., 2004. **351**: p. 2159-2169.
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7. Johansson, M., D.G. Denardo, and L.M. Coussens, *Polarized immune responses differentially regulate cancer development.* Immunol. Rev., 2008. **222**: p. 145-154.
8. Montovani, A., A. Sica, and M. Locati, *New vistas on macrophage differentiation and activation.* Eur. J. Immunol., 2007. **37**: p. 14-16.
9. Miselis, N.R., et al., *Targeting tumor-associated macrophages in an orthotopic murine model of diffuse malignant mesothelioma.* Mol. Cancer Ther., 2008. **7**(4): p. 788-799.

## VII. APPENDICES

A. Complete academic curriculum vitae for Dr. V. Courtney Broaddus and Dr. Lisa Coussens
B. Publications for months 1-12
C. Abstracts for months 1-12

July 2010

**UNIVERSITY OF CALIFORNIA SAN FRANCISCO**
**CURRICULUM VITAE**

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## EDUCATION:

| | | | |
|---|---|---|---|
| 1971-1975 | Duke University, Durham, NC | B.S. | Summa Cum Laude, Zoology |
| 1975-1979 | University of Pennsylvania | M.D. | Medicine |
| 1979-1980 | University of Pennsylvania | Intern | Medicine |
| 1980-1982 | University of Pennsylvania | Resident | Medicine |
| 1983-1986 | University of California, San Francisco | Fellow | Pulmonary/ Critical Care Medicine |
| 2001-2002 | Comprehensive Cancer Center, UCSF | Sabbatical | Laboratory of Gerard Evan, Ph.D. |
| 2007 | Leadership Development for Physicians in Academic Health Centers | | Harvard School of Public Health |

## LICENSING AND CERTIFICATION

| | | |
|---|---|---|
| 1982 | California License | G-049379 |
| 1982 | Internal Medicine | American Board of Internal Medicine |
| 1986 | Pulmonary Disease | American Board of Internal Medicine |
| 1999-2009 | Critical Care Medicine | American Board of Internal Medicine |

## PRINCIPAL POSITIONS HELD

| | | | |
|---|---|---|---|
| 1986-1988 | University of California, SF | Instructor in Residence | Medicine |
| 1988-1995 | University of California, SF | Assistant Professor in Residence | Medicine |
| 1995-1997 | University of California, SF | Associate Professor in Residence | Medicine |
| 1997-2001 | University of California, SF | Associate Professor | Medicine |
| 2001-now | University of California, SF | Professor | Medicine |

## OTHER POSITIONS HELD CONCURRENTLY

| | | |
|---|---|---|
| 1998-now | Dept of Medicine | Chief, Division of Pulmonary and Critical Care Medicine, SFGH |
| 2004-now | Dept of Medicine | Associate Director, Lung Biology Center, SFGH |

## HONORS AND AWARDS

| | |
|---|---|
| 1974 | Phi Beta Kappa, Junior Year |
| 1975 | Summa Cum Laude |
| 1978 | Alpha Omega Alpha, Junior Year |
| 1979 | Janet M.Glasgow Memorial Award and Citation |
| 1985 | Individual National Research Service Award, NIH |
| 1987 | Clinical Investigator Award, NHLBI |
| 1988 | American Physiological Society, Elected |
| 1991 | Distinction in Teaching Award, Academic Senate, UCSF |
| 1991 | Associate, Scientific Staff, Cardiovascular Research Institute, Elected |
| 1992 | Pre-tenure Award, UCSF |
| 1999-2002 | President, International Mesothelioma Interest Group |
| 2003 | Nomination for Most Outstanding Teacher, UCSF Women In Medicine |
| 2005-now | Best Doctors in America, Selected |
| 2005 | Faculty of the Year Award, SFGH Association of Business Officers' Group |
| 2006 | Western Society of Clinical Investigation, Elected |
| 2007&2008 | Nomination for Subspecialist Consultant of the Year Award, SFGH |
| 2008 | Nomination for Wagner Award, International Mesothelioma Interest Group |
| 2010 | Michael S. Stulbarg Outstanding Teaching Award, UCSF Pulmonary |
| 2010 | Pioneer Award, The Mesothelioma Applied Research Foundation. |

## KEYWORDS/AREAS OF INTEREST

Apoptosis, mesothelioma, macrophages, synergy, 3-dimensional models, TRAIL (TNF-related apoptosis-inducing ligand), pleural disease, pleural effusions, asbestos.

## PROFESSIONAL ACTIVITIES

CLINICAL

Attending, Medical Intensive Care Unit, SFGH: I attend 1 month of the year on the ICU, 7 days a week, supervising 8 senior residents and interns.

Attending, Pulmonary Consult Service, SFGH: I attend 1 month each year seeing inpatients with pulmonary problems, 5 days a week, supervising 2 fellows and 1-2 residents/medical students.

## PROFESSIONAL ORGANIZATIONS

Memberships
Lung Biology Center, San Francisco General Hospital
Thoracic Oncology Research Group, UCSF Cancer Center
Cardiovascular Research Institute, University of California, San Francisco
Western Society of Clinical Investigation
American Thoracic Society
California Thoracic Society
International Mesothelioma Interest Group
Mesothelioma Applied Research Foundation
International Association for the Study of Lung Cancer (IASLC)

Service to Professional Organizations

| | | |
|---|---|---|
| 1988-1992 | ALA/American Thoracic Society | Research Fellowship Review Committee |
| 1989-1991 | ALA/American Thoracic Society | Manpower Review Committee |
| 1991-1992 | American Thoracic Society | Program Committee, Respiratory Structure and Function |
| 1993-1994 | American Thoracic Society | Nominating Committee, Respiratory Cell and Molecular Biology |
| 1994-1995 | American Thoracic Society | Women's Affairs Committee |
| 1994-1997 | American Thoracic Society | Program Committee, Respiratory Cell and Molecular Biology |
| 1995-1997 | California Lung Association | Research Fellowship Committee |
| 1995-2001 | American Thoracic Society | Long Range Planning Committee, Respiratory Cell and Molecular Biology |
| 1995-1997 | American Thoracic Society | Program and Budget Committee |
| 1995-1999 | Am Federation for Med Research | UCSF Representative |
| 1997-2003 | American Lung Association of CA | Research Administrative Committee |
| 1999-2002 | Intl Mesothelioma Interest Group | President |
| 1999-2001 | American Thoracic Society | Chair, Long Range Planning Committee, Respiratory Cell and Molecular Biology |
| 2000-2002 | American Thoracic Society | Planning on ATS Research Agenda |
| 2000-2002 | American Thoracic Society | Assembly Structure Task Force |
| 2003-2004 | American Thoracic Society | Education Committee |
| 2003-2007 | American Thoracic Society | Scientific Advisory Committee |
| 2005-now | International Mesothelioma Interest Group | Member, Board of Directors<br>Scientific Organizing Committee |
| 2006-now | Intl Mesothelioma Interest Group | Chair, Website Subcommittee |
| 2007-2009 | American Thoracic Society | Chair, Nominating Committee, Respiratory Cell and Molecular Biology |
| 2007-2008 | American Thoracic Society | Member, Search Committee for Editor, Am. Journal of Respiratory Cell Mol. Biol. |
| 2008-now | Mesothelioma Applied Research Foundation | Member, Scientific Advisory Board |

**SERVICE TO PROFESSIONAL PUBLICATIONS**

1990-now Ad hoc referee for:
Oncogene (2 papers in last year)
American Journal of Pathology (1 paper in last year)
New England Journal of Medicine (2 papers in last year)
American Journal of Respiratory and Critical Care Medicine (4 papers in 2 years)
American Journal of Respiratory Cell and Molecular Biology (6 papers in 2 years)

1996-1998 Associate Editor,
American Journal of Respiratory Cell and Molecular Biology

2000-2007 Editorial Board, American Journal of Physiology:
Lung Cellular and Molecular Physiology

2002-2006 Editor, Murray and Nadel's Textbook of Respiratory Medicine 4th Edition
2007-now Editor, Murray and Nadel's Textbook of Respiratory Medicine 5th Edition
2005-now Editor, Website for Murray and Nadel's Textbook of Respiratory Medicine
www.respmedtext.com

2010-2013 Editorial Board, Apoptosis

**INVITED PRESENTATIONS**

INTERNATIONAL

International Chemokine Symposium, Bath, England, 1995 (Invited speaker)
International Meeting on the Toxicology of Natural and Man-Made Fibrous and Non-Fibrous Particles, Lake Placid, NY, 1996 (Platform)
International Meeting of the Formosan Medical Association, Taiwan, 1999 (Platform)

American Thoracic Society International Conference. 1986 (Plenary talk); 1988 (Invited speaker, Course on the Academic Pulmonary Physician); 1989 (Invited speaker, Forum on Training and Transition); 1992 (Co-Chair, Poster Symposium); 1993 & 1994 (Co-Chair, Poster Discussion Symposium, Symposium); 1994 (Invited Speaker, Symposium); 1995 (Invited Speaker, Symposium; Meet the Professor Seminar); 1995 & 1997 (Chair, Speaker, Mini-symposium); 1996 (Co-chair, Speaker, Minisymposium); 1997 (Featured speaker, Minisymposium); 1999 (Invited speaker); 2000 (Speaker); 2001 (Co-chair, speaker, Mini-symposium); 2003 (Chair, Symposium); 2004 (Co-chair, Symposium; Meet the Professor Seminar); 2005 (Invited plenary speaker); 2006 (Invited Speaker, Participant in Expert Clinician Panel); 2007 (Co-Chair, Symposium; Presenter, Trudeau Award; Participant in Master Clinician Panel); 2008 (Participant in Master Clinician Panel; Co-Chair Symposium; Presenter); 2009 (Invited Clinical Expert in Pleural Disease; Participant in Master Clinician Panel; Co-Chair, Minisymposium)

International Mesothelioma Interest Group. San Francisco, 1993 (Invited Speaker); Paris, 1995 (Co-organizer, speaker); United Kingdom, 1999 (Speaker); Brescia, Italy, 2003 (Co-organizer, Speaker); Chicago, 2006 (Organizer Apoptosis Satellite Session, Co-chair, speaker). 2008 (Invited Plenary Speaker, Co-chair of symposium), 2009 (Program Planning Executive Committee, Speaker, Chair Symposia)

NATIONAL

1988 Advances in Internal Medicine, San Francisco, CA
1989 Meet the Professor, American College of Physicians, San Francisco, CA
1989 University of Oklahoma Health Science Center, Oklahoma City, OK
1989 St. Luke's-Roosevelt Hospital Center, Columbia University, New York, NY
1990 National Institutes of Health Workshop, Bethesda, MD
1990 Invited Speaker, Scientific Conference on Acute Lung Injury, American Heart Association, Dallas, TX
1990 Advances in Internal Medicine, San Francisco, CA
1991 Advances in Internal Medicine, San Francisco, CA
1994 Chair and Invited Speaker, Cambridge Health Institute Conference on Inflammatory Cytokine Antagonists, Philadelphia, PA
1994 Invited Speaker, University of Pennsylvania, Philadelphia, PA
1994 Gordon Conference on Chemotactic Chemokines
1994 University of Pennsylvania, Philadelphia, PA
1995 Texas Thoracic Society, Austin, TX
1996 Selected Participant, Professional Development Seminar for Senior Women in Medicine, Association of American Medical Colleges, Washington DC
1996 Co-Moderator, Pleural Disease Minisymposium. American College of Chest Physicians, San Francisco, CA
1997 Visiting Pulmonary Scholar, Duke-UNC-NCSU-NIEHS-EPA-CIIT, NC
1998 Visiting Speaker, St. Thomas Hospital, Vanderbilt Univ, Nashville, TN
1998 Invited Speaker, First Annual Symposium on the Pleura, St. Thomas Hospital, Vanderbilt, TN.

1998 Visiting Professor, Medical Grand Rounds, University of Texas at Tyler, TX.
2000 Visiting Professor, Stanford University, Palo Alto, CA
2000 Invited Participant and Co-Chair, American Thoracic Society-
National Institute of Environmental Health Science (ATS-NIEHS)
Workshop-Toronto, Canada
2002 Visiting Professor, University of Southern California, Los Angeles, CA
2002 Visiting Professor, Yale University, New Haven, CT
2003 Visiting Professor, University of Montana,
Center for Environmental Health Sciences, Missoula, MT
2004 Visiting Professor, University of Pennsylvania, Philadelphia, PA
2006 Co-Chair, Minisymposium, Experimental Biology, San Francisco, CA.
2006 Invited Participant and Co-Chair, International Mesothelioma Interest Group,
Apoptosis Satellite Symposium, Chicago, IL
2006 Invited Speaker, University of Chicago School of Medicine
2006 Visiting Professor, Northwestern University School of Medicine
2007 Invited Speaker, International Mesothelioma Program,
Harvard Medical School/Brigham & Women's Hospital, Boston, MA
2008 Invited Speaker, International Mesothelioma Interest Group, Amsterdam
2009 Invited Speaker, NIH Mesothelioma Symposium, Bethesda, MD.
2009 Invited Speaker, ATS/Hawaii Thoracic Society State of the Art Course,
Maui, HI
2009 Invited Outside Reader, PhD Dissertation, Bonnie Lau, Dept of Pathology,
Brown University, Providence, RI
2009 Invited Speaker, International Mesothelioma Program,
Workshop on Preclinical Drug and Target Discovery,
Brigham and Womens Hospital, Boston, MA
2010 Invited Speaker, Mesothelioma Applied Research Foundation,
Washington, DC.

REGIONAL AND OTHER INVITED PRESENTATIONS

1988 Medical Grand Rounds, SFGH
1993 Research Conference on Lung Injury, Genentech, So SF
1994 SFGH Cellular and Molecular Medicine Seminar
1995 Medical Grand Rounds, VAMC/ SFGH
1996 Medical Grand Rounds, Moffitt/UCSF
1996 Selected Participant, Senior Women's Conference,
University of California San Francisco
1996 Panel Discussant, UCSF Department of Medicine.
1996 Medical Grand Rounds, SFGH
1997 Invited Speaker, UCSF Pulmonary Retreat, Asilomar, CA.
1998 Invited Speaker, UCSF Pulmonary Retreat, Asilomar, CA.
1998 Invited Speaker, Thoracic Oncology Conference, UCSF/Stanford/Mt. Zion
2000 Medical Grand Rounds, Stanford University
2000 Invited speaker, Division of Pulmonary and Critical Care, Stanford Univ.
2000 Invited Speaker, Pulmonary Research Retreat, UCSF
2003 Medical Grand Rounds, SFGH
2003 Invited Speaker, Recent Advances in Pulmonary and Critical Care Medicine
2003 Invited Speaker, Pulmonary Research Retreat, UCSF
2005 Invited Speaker, Thoracic Oncology Research Group, UCSF
2005 Invited Speaker, Radiation Oncology Grand Rounds, UCSF and Mt Zion
2006 Invited Speaker, Thoracic Oncology Research Group, UCSF
2006 Invited Speaker, Pulmonary Research Retreat, UCSF
2008 Invited Speaker, Dean's Seminar Series, SFGH
2009 Invited Speaker, Pulmonary Research Retreat, UCSF

## GOVERNMENT AND OTHER PROFESSIONAL SERVICE

1994 Ad Hoc Member, Comparative Medicine Review Committee, NIH
1999 Ad Hoc Reviewer, National Heart, Lung, and Blood Institute
2001 Ad Hoc Reviewer, National Cancer Institute
2002 Invited Member, NIH Center for Scientific Review, Special Emphasis Panel, Experimental Therapeutics Panel (ZRG1 ET-1).
2004 Site Reviewer, Program Project Grant Review Committee, NCI. MGH/Brigham and Women's, Boston, MA
2005 Invited Member, National Asbestos Research Working Group National Health and Medical Research Council, Australia
2006 Invited Participant, NHLBI Strategic Plan for the Division of Lung Diseases
2009 Reviewer, FY09 Peer-reviewed Medical Research Program, AIBS, US Army Medical Research and Materiel Command
2009-now Expert Panel Member, NIEHS Asbestos Mechanism of Action Workshop

## UNIVERSITY AND PUBLIC SERVICE
## UNIVERSITY SERVICE

UCSF CAMPUS-WIDE

1996-1998 Member, Chancellor's Award for the Advancement of Women Committee
2003-now Member, Academic Senate Committee on Research, UCSF
2005 Chair, Academic Senate Task Force Reviewing University of California Policy on Human Subject Injury
2006-2007 Member, Reconvened SFGH Subcommittee of the Chancellor's Advisory Committee on the Long Range Planning Amendment
2006 & 2007 Faculty Presenter, Inaugural Faculty Welcoming Week, Chancellor's Council on Faculty Life, "Building a Research Career"
2007 Small group leader, Junior faculty retreat, Striving & Thriving in the Academic World, Chancellor's Advisory Committee on the Status of Women

SCHOOL OF MEDICINE

1988-1989 Internship Selection Committee, Univ. of Calif, San Francisco
2005 Member, Stewardship Review Committee, Chair of Dermatology, UCSF
2005 Member, Ad Hoc Subcommittee on Faculty Misconduct, Office of Academic Affairs
2006- 2007 Member, Search Committee for Chair, Department of Medicine
2006- 2007 Member, Select Subcommittee of Search Committee for Chair, Department of Medicine, UCSF
2007 Invited speaker, The Senior Faculty Career Challenge, Dean's Office of School of Medicine. “Strategies for revitalizing divisional goals/function”
2007- now Member, Scholarships and Awards Committee, UCSF School of Medicine

DEPARTMENT OF MEDICINE

1988-1989 Member, Bylaws Committee, San Francisco General Hospital
1989 Member, Program Planning Committee Recent Advances in Pulmonary & Critical Care Medicine (8th Annual), University of California, San Francisco
1989-2000 Member, Critical Care Committee, San Francisco General Hospital
1990-1991 Chair, Program Planning Recent Advances in Pulmonary & Critical Care Medicine (9th and 10th Annual), Univ of California, San Francisco

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| 1990-present | Member, Steering Committee of the Pulmonary Research Group, University of California, San Francisco (elected) |
| 1993-present | Member, Pulmonary Fellowship Selection Committee, University of California, San Francisco |
| 1994-present | Member, Pulmonary Research Group Retreat Planning Committee |
| 1994-1996 | Member, Search Committees for Chief of Surgical Research and Chief of Rheumatology, San Francisco General Hospital |
| 1996-1998 | Member, Task Force on Diversity, University of California, SF |
| 1996-1999 | Member, Search Committee for Joint Appointment in Radiology/Pulmonary and Critical Care Medicine, San Francisco General Hospital |
| 1996-2002 | Member, Promotions Subcommittee, Academic Senate, University of California, San Francisco |
| 1996-1997 | Member, Executive Committee, Department of Medicine, SFGH |
| 1996-2002 | Board Member, UCSF/Macy's Center for Creative Therapies, San Francisco General Hospital |
| 1997 | Member, Search Committee, first Associate Chair for Biomedical Research, Department of Medicine, UCSF |
| 1997-1998 | Member, Search Committee for Chief of Cardiology, SFGH |
| 1998-1999 | Member, Search Committee for Chief of Gastroenterology, SFGH |
| 1998-1999 | Member, Search Committee, Chief, Pulmonary and Critical Care Med, UCSF |
| 1998-2000 | Member, General Clinical Research Center Advisory Committee |
| 1999-2000 | Member, Search Committee for Thoracic Surgeon, SFGH |
| 2000 | Member, Search Committee for Chief of Radiology, SFGH |
| 2000 | Member, Search Committee for Manager, Department of Medicine, SFGH |
| 2003-2004 | Chair, Search Committee Chief of Pulmonary & Critical Care Medicine, Veteran's Administration Medical Center, UCSF |
| 2005-2006 | Member, Search Committee for Chest Radiologist, SFGH |
| 2005 | Member, John Carbone Chair Nominating Committee |
| 2006- | Member, Search Committee for Scientist, Surgical Research Laboratory |
| 2006-2007 | Member, Search Committees for Gastroenterology FTE |
| 2006-current | Chair, Committee to Establish John F. Murray Distinguished Professorship |
| 2008-now | Member, Search Committee for Lung Biology Center Physician-Scientist |
| 2008-now | Member, Search Committee for Chief, Division of Pulmonary & Critical Care Medicine, UCSF |
| 2009-now | Chair, Search Committee for Pulmonary Faculty Member, SFGH/UCSF. |
| 2009-now | Member, Recruitment and Retention Workgroup, Department of Medicine Strategic Plan |

**PUBLIC SERVICE**

| | |
|---|---|
| 1997 | Presenter on Asbestos-Related Diseases, Gloria R. Davis Academic Middle School Asbestos Exposure & Risk Assessment, S.F. Department of Public Health |
| 1997 | Coordinator, Cigarette Smoke Demonstration, Take Your Daughters to Work Day |
| 2005-7 | Lecturer on Avian and Mammal Lungs, San Francisco Day School |
| 2008-9 | Fundraiser, Annual Fund, International High School, San Francisco. |
| 2009-now | Class Captain, Annual Fund, International High School, San Francisco. |

## TEACHING AND MENTORING

Other Courses

| | |
|---|---|
| 1990-now | Pulmonary Physiology Seminars (2 hours/year) |
| 1990 | Faculty Leader, Preparing Interns for Residency |
| 1990-now | Medical Service Conferences |
| 1991 | Women's Medical Student Association Retreat |
| 1993-2003 | Lecturer to Medical Residents (Parnassus, SFGH, VAMC) |
| 1987-2002 | Speaker, Recent Advances in Pulmonary & Critical Care Medicine |
| 1998-now | Summer Seminar Series and Practical Sessions on Pleural Disease |
| 2004 | Faculty Coach and Presenter for Junior Faculty, Mid-Term Appraisal for Faculty at UCSF |
| 2004 | Annual PIBS/BMS Course, Ethics and the Responsible Conduct of Research |
| 2005 | Lecturer to Medical Residents (Parnassus, SFGH, VAMC) |
| 2005-2007 | Workshop Leader, Mid-term Appraisal for Faculty at UCSF |

Predoctoral Students Supervised

| *Dates* | *Name* | *Position while supervised* | *Current position* |
|---|---|---|---|
| 1995-1997 | Sudha Rani Narasimha | Medical Student | Medical Resident, UCLA |
| 1997-1999 | Jack Wu | Undergraduate | Medical resident Cook County, Chicago, IL |
| 2002-2004 | Kevin Lee | Undergraduate | Medical student |
| 2009- now | Nikita Kolhatkar | Graduate | current |

Graduate Students Supervised

| *Dates* | *Name* | *Position while supervised* | *Current position* |
|---|---|---|---|
| 2005-now | Dario Barbone | Graduate student | Postdoctoral scholar, UCSF |
| 2008-9 | Bonnie Lau | Graduate student | MD/PhD Brown University |

Postdoctoral Fellows Supervised

| *Dates* | *Name* | *Position while supervised* | *Current position* |
|---|---|---|---|
| 1989-1991 | Alice M. Boylan, MD | Pulmonary research fellow | Associate Professor of Medicine Medical Univ of South Carolina |
| 1991-1993 | Rex Yung, MD | Pulmonary research fellow | Associate Professor of Medicine Johns Hopkins Univ. |
| 1993-1995 | Hans Folkesson, PhD | Postdoctoral research fellow | Associate Professor of Physiology Northeastern Ohio University, OH |
| 1995-1996 | Jamie Bigelow, MD | Pulmonary research fellow | Pulmonologist St. Francis Hospital, SF |
| 1996-1997 | Evaldo Marchi, MD | Visiting research fellow | Professor of Surgery Sao Paolo, Brazil |
| 1998-2000 | Tom Geiser, MD | Postdoctoral research fellow | Associate Professor of Medicine University Hospital, Bern, SW |
| 2000-2001 | Masa Ishigaki, MD PhD | Visiting research fellow | Associate Professor, Japan |
| 2000-2003 | Claire Vivo, PhD | Postdoctoral research fellow | Research Scientist Ordway Research Institute, NY |
| 2003-2004 | Ki Up Kim, MD | Visiting research fellow | Associate Professor of Medicine Soonchunhyang University Hospita South Korea |
| 2004-2005 | Lorriana Leard, MD | Pulmonary research fellow | Assistant Prof of Medicine, UCSF |
| 2003-2006 | Keith Abayasiriwardana, PhD | Postdoctoral research fellow | Research Scientist, Merck Laboratories, London, UK |

| | | | |
|---|---|---|---|
| 2006- 2008 | Tsung-Ming Yang, MD | Postdoctoral research fellow | Assistant Professor,<br>Chiayi Chung-Gung Memorial Hosp<br>Chiayi, Taiwan |
| 2008-on | Eunice Kim, MD | Pulmonary Fellow | Pulmonary Research Fellow |
| 2008-on | Denitza Blagev, MD | Pulmonary Fellow | Pulmonary Research Fellow |
| 2008-on | Joyce Lee, MD | Pulmonary Fellow | Pulmonary Research Fellow |
| 2009-on | Joshua Galanter, MD | Pulmonary Fellow | Pulmonary Research Fellow |

INFORMAL TEACHING

1987-now: Attending Rounds, Pulmonary Consult Service, SFGH
(1 month/year with 2 fellows, 1 medical student and/or resident)

1987-now: Attending Rounds, Medical ICU Service, SFGH
(1.5 month/year with 4 3rd year residents and 4 interns/ informal and formal teaching)

1999-2002 Attending Rounds, Medicine Service, SFGH

**FACULTY MENTORING**

I have selected some representative examples of mentoring relationships from recent years.

| *Dates* | *Name* | *Position While Mentoring* | *Role* | *Current Position* |
|---|---|---|---|---|
| 2000-2002 | David Morris, MD | Division chief | Informal advisor | Head Respiratory Research<br>Roche, Palo Alto, CA |
| 2000-2005 | Robert Jasmer, MD | Division chief | Advisor | Physician, private practice |
| 2004-now | Payam Nahid, MD | Division chief | Advisor, Reviewed grants | Asst Prof, UCSF |
| 2004-now | Mary Gray, MD | LBC Assoc Director | Recruited into LBC, collaborator/advisor | Assoc Prof, UCSF |
| 2002-now | Laura Koth, MD | LBC Assoc. Dir. | Career advisor | Asst Prof, UCSF |
| 2004-now | Lorriana Leard, MD | Lab head/Div.chief | Advisor, formal mentor | Asst Prof, UCSF |
| 2005-2007 | Dana McClintock, MD | Career committee | Selected formal mentor | Pulmonary fellow |
| 2005-now | Harold Collard, MD | Division chief | Advisor, reviewed grants | Asst Prof, UCSF |
| 2006-now | Janet Diaz, MD | Division chief | Advisor | Asst Prof, UCSF |

**TEACHING AWARDS AND NOMINATIONS:**

1991 Distinction in Teaching Award, Academic Senate, UCSF
2003 Nomination for Most Outstanding Teacher, UCSF Women In Medicine
2007 & 2008 Nominations for Subspecialist Consultant of the Year Award, SFGH Dept of Medicine

**SUMMARY OF TEACHING HOURS:**

2007-2008 Total anticipated hours of teaching: 540
Formal class or course teaching hours: 20
Informal teaching hours: 250
Mentoring hours: 270

2008-2009 Total anticipated hours of teaching: 520
Formal class or course teaching hours: 20
Informal teaching hours: 250
Mentoring hours: 250

2009-2010 Total anticipated hours of teaching: 540
Formal class or course teaching hours: 20
Informal teaching hours: 250
Mentoring hours: 270

## RESEARCH AND CREATIVE ACTIVITIES

### RESEARCH AWARDS AND GRANTS

**ACTIVE**:

**Supporting Agency:** Peer-Reviewed Medical Investigator-Initiated Research Program – Department of Defense
**Grant Title:** *The Role of Macrophage-induced Inflammation in Mesothelioma.*
**Role on project :** Principal Investigator
**Time Commitment to Project**: 3 Calendar Months Effort (25%)
**Total Funding Period:** 04/01/2009 – 3/31/2012 **Direct Dollars: $300,000 / yr 1**
**$900,000 / yr 1-3**
**Project Overlap:** None
**Goals:** To demonstrate the functional significance of macrophages as promoters of tumor cell survival in mesothelioma and determine whether tumor-associated macrophages can be repolarized to enhance mesothelioma cell apoptosis.
**Specific Aims:** 1. To determine the functional significance of macrophage phenotype in mesothelioma. 2. To determine the functional significance of macrophages as regulators of mesothelioma apoptosis in vitro. 3. To define the functional significance of macrophage depletion or repolarization on mesothelioma survival in vivo.

**Supporting Agency:** Ireland- NCI Cancer Consortium
**Total Funding Period:** 1/1/2009 – 12/31/2011
**Grant Title**: *Role of the Bcl-2 Family in Apoptotic Resistance of Mesothelioma to Bortezomib.*
**Role on project**: Co-Principal Investigator
**Time Commitment to Project**: No time **Direct Dollars**: $135,000 / yr

**Program Overlap**: None
**Goals**: To support a post-doctoral fellow to determine the mechanism of apoptotic resistance in 3 dimensional models of mesothelioma.
**Specific Aims**: 1) To study the mitochondrial anti-apoptotic repertoire that determines resistance of mesothelioma to bortezomib. 2) To uncover the role of the mitochondrial anti-apoptotic repertoire that contributes to the acquired apoptotic resistance in 3D models to bortezomib.

**Supporting Agency:** NCI
**Grant mechanism:** RO1 CA135358-01
**Total Funding Period:** 8/11/2008 – 5/31/2012
**Grant Title**: *Targeted Liposomal Radiotherapy of Malignant Mesothelioma*
**Role on project**: Co-Investigator
**Time Commitment to Project**: 5%

**Program Overlap**: None
**Goals**: To engineer nanoliposomes as the dose delivery media to carry high payload of radionuclides for specific targeting of the mesothelioma tumor cells.
**Specific Aims**: 1) To identify optimal scFv antibodies targeting to malignant mesothelioma from a panel of 15 antibodies we have recently discovered. The native antibodies will be labeled with 99mTc for in vitro and in vivo. 2) To focus on the 177Lu labeling of the nanosized liposome conjugated with optimal antibody (177Lu-LP-scFv) to achieve high specific activity and characterize the targeting capability, stability and radiolysis in vitro 3) To determine the biodistribution and treatment efficacy of the best 177Lu-LP-scFv in mice with malignant peritoneal mesothelioma xenografts.

***Past***

| | |
|---|---|
| NIH Institutional National Research Service Award (HL07185) | 1983-1985 |
| NIH Individual National Research Service Award (HL07271) | 1985-1986 |
| Academic Senate Committee on Research Grant<br>Origin of pleural effusions in volume-loaded sheep | 1986 |
| Academic Senate Committee on Research Grant<br>Origin of pleural effusions in hydrostatic pulmonary edema | 1988 |
| NHLBI Pulmonary Vascular SCOR (HL19155) (PI)<br>Dynamics of pleural liquid turnover in health and disease. | 1986-1991 |
| American Lung Association Research Grant,<br>Formation of pleural effusions in pleural inflammation. | 1987-1989 |
| Clinical Investigator Award, NHLBI (KO8 HL01893)<br>Comparative physiology of the normal and inflamed pleura. | 1987-1992 |
| NHLBI Pulmonary Vascular SCOR (HL19155) (Co-Investigator)<br>Mechanisms of acute pleural and lung injury. | 1991-1993 |
| Pretenure Award, University of California, San Francisco<br>Mechanisms of interaction of asbestos and mesothelial cells. | 1992-1993 |
| Corvas International, San Diego, CA. (Co-Investigator)<br>The role of rabbit IL-8 in neutrophil-mediated inflammation. | 1993-1994 |
| Genentech (PI)<br>The role of rabbit IL-8 in neutrophil-mediated inflammation. | 1993-1994 |
| RO1 ES06331 (NIEHS) (PI)<br>Molecular interactions of asbestos and mesothelial cells. | 1994-1998 |
| Genentech (PI)<br>The role of rabbit IL-8 in sepsis. | 1995-1997 |
| Research Evaluation and Allocation Committee Grant, UCSF<br>The role of fiber internalization in mediating the toxic effects of asbestos on mesothelial cells. | 1996-1997 |
| Principal Investigator, Tobacco-related Disease Research Program<br>Programmed cell death in cigarette-induced lung disease. | 1998-2001 |
| RO1 ES08985 (NIEHS) (PI)<br>Protective role of apoptosis in asbestos pleural injury. | 1997-2002 |
| RO1 ES08985 (NIEHS) (PI)<br>Supplement for microarray studies. | 2000-2002 |
| Peterson Family Foundation (PI)<br>Role of Akt/mTOR in mesothelioma. | 2005-2007 |
| Buzzi Foundation, Italy<br>Role of PI3K/Akt/mTOR pathway in resistance to apoptosis. | 2005-2007 |
| RO1 CA95671 (NIH/NCI) (PI)<br>Amplification of TRAIL-induced apoptosis in mesothelioma. | 2003-2009 |
| Mesothelioma Applied Research Foundation Award.<br>Antibody development against mesothelioma. | 2007-2009 |

**PEER REVIEWED PUBLICATIONS**


1. BROADDUS C, Dake M, Stulbarg MS, Blumenfeld W, Hadley K, Golden JA, Hopewell PC. Bronchoalveolar lavage and transbronchial biopsy for the diagnosis of pulmonary infections in patients with the acquired immunodeficiency syndrome. Ann Intern Med 102:747-752, 1985.

2. Wiener-Kronish JP, Goldstein R, Matthay RA, Biondi JW, BROADDUS VC, Chatterjee K, Matthay MA. Lack of association of pleural effusion with chronic pulmonary arterial and right atrial hypertension. Chest 92:967-970, 1987.

3. BROADDUS VC, Wiener-Kronish JP, Berthiaume Y, Staub NC. Removal of pleural liquid and protein by lymphatics in awake sheep. J Appl Physiol 64:384-390, 1988.

4. Berthiaume Y, BROADDUS VC, Gropper MA, Tanita T, Matthay MA. Alveolar liquid and protein clearance from normal dog lungs. J Appl Physiol 65:585-593, 1988.

5. Wiener-Kronish JP, BROADDUS VC, Albertine KH, Gropper MA, Matthay MA, Staub NC. Relationship of pleural effusions to increased permeability pulmonary edema in anesthetized sheep. J Clin Invest 82:1422-1429, 1988.

6. BROADDUS VC, Wiener-Kronish JP, Staub NC. Clearance of lung edema into the pleural space of volume-loaded anesthetized sheep. J Appl Physiol 68:2623-2630, 1990.

7. BROADDUS VC, Araya M, Carlton DP, Bland RD. Developmental changes in pleural liquid protein concentration in sheep. Am Rev Resp Dis 143:38-41, 1991.

8. Jacobson MA, Mills J, Rush J, Peiperl L, Seru V, Mohanty PK, Hopewell PC, Hadley WK, BROADDUS VC, Leoung G, Feigal DW. Morbidity and mortality of patients with AIDS and first-episode *Pneumocystis carinii* pneumonia unaffected by concomitant pulmonary cytomegalovirus infection. Am Rev Respir Dis 144:6-9, 1991.

9. BROADDUS VC, Araya M. Liquid and protein dynamics using a new, minimally invasive pleural catheter in rabbits. J Appl Physiol 72:851-857, 1992.

10. Boylan AM, Rüegg C, Hoeffel J, Kim KJ, Hébert CA, Pytela R, Sheppard D, Goldstein IM, BROADDUS VC. Evidence of a role for mesothelial cell-derived interleukin-8 in the pathogenesis of asbestos-induced pleurisy in rabbits. J Clin Invest 89:1257-1267, 1992.

11. BROADDUS VC, Hébert CA, Vitangcol RV, Hoeffel JM, Bernstein MS, Boylan AM. Interleukin-8 is a major neutrophil chemotactic factor in pleural liquid of patients with empyema. Am Rev Respir Dis 146:825-830, 1992.

12. BROADDUS VC, Feigal DW Jr. Starting an academic career: a survey of junior academic pulmonary physicians. Chest 105:1858-1863, 1994.

13. BROADDUS VC, Hoeffel JM, Boylan AM, Sadick M, Chuntharapai A, Kim KJ, Hébert CA. Neutralization of interleukin-8 inhibits neutrophil influx in a rabbit model of endotoxin-induced pleurisy. J Immunol 152:2960-2967, 1994.

14. Boylan AM, Hébert CA, Sadick M, Wong WL, Hoeffel JM, Hartiala KT, BROADDUS VC. Interleukin-8 is a major component of pleural liquid chemotactic activity in a rabbit model of endotoxin pleurisy. Am J Physiol Lung Cell Mol Physiol 267(11):L137-L144, 1994.

15. Folkesson HG, Matthay MA, Hébert CA, BROADDUS VC. Acid aspiration lung injury in rabbits is mediated by interleukin-8 dependent mechanisms. J Clin Invest 96:107-116, 1995.

16. Boylan AM, Sanan DA, Sheppard D, BROADDUS VC. Vitronectin enhances internalization of crocidolite asbestos by rabbit pleural mesothelial cells via the integrin v 5. J Clin Invest 96:1987-2001, 1995.

17. BROADDUS VC, Yang L, Scavo LM, Ernst JD, Boylan AM. Asbestos induces apoptosis of human and rabbit pleural mesothelial cells via reactive oxygen species. J Clin Invest 98:2050-2059, 1996. (* *identified by the Editors as being of broad interest*)

18. BROADDUS VC, Yang L, Scavo LM, Ernst JD, Boylan AM. Crocidolite asbestos induces apoptosis of pleural mesothelial cells: Role of reactive oxygen species and poly (ADP-ribosyl) polymerase. Environ Health Perspect 105 (Suppl 5):1147-1152, 1997.

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25. Levresse V, Renier A, Levy F, BROADDUS VC, Jaurand M-C. DNA breakage in asbestos-treated normal and transformed (TSV40) rat pleural mesothelial cells. Mutagenesis 15(3): 239-244, 2000.

26. Liu W, Ernst JD, BROADDUS VC. Phagocytosis of crocidolite asbestos induces oxidative stress, DNA damage and apoptosis in mesothelial cells. Am J Respir Cell Mol Biol 23(3): 371-378, 2000.

27. Wu J, Liu W, Koenig K, Idell SI, BROADDUS VC. Vitronectin adsorption to chrysotile asbestos increases phagocytosis and toxicity for mesothelial cells. Am J Physiol Lung Cell Mol Physiol 279:L916-L923, 2000.

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35. Kim KU, Wilson SM, Abayasiriwardana K, Collins R, Fjellbirkeland L, Xu Z, Jablons DM, Nishimura SL, BROADDUS VC. A novel in vitro model of human mesothelioma for studying tumor biology and apoptotic resistance. Am J Respir Cell Mol Biol 33(6):541-8, 2005.

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(* *equal contributors as senior author*)

39. Abayasiriwardana KS, Barbone D, Kim KU, Vivo C, Lee KK, Dansen TB, Hunt AE, Evan GI, BROADDUS VC. Malignant mesothelioma cells are rapidly sensitized to TRAIL-induced apoptosis by low dose anisomycin via Bim. Mol Cancer Ther 6(10):2766-2776, 2007.

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44. Bidlingmaier S, He J, Wang Y, An F, Feng J, Barbone D, Gao D, Franc B, BROADDUS VC, Liu B. Identification of MCAM/CD146 as the target antigen of a human monoclonal antibody that recognizes both epithelioid and sarcomatoid types of mesothelioma. Cancer Res 69(4); 1570-1577, 2009.

45. Yang TM, Barbone D, Fennell DA, BROADDUS VC. Bcl-2 family proteins contribute to apoptotic resistance in lung cancer multicellular spheroids. Am J Respir Cell Mol Biol 41(1):14-23, 2009.

(* With accompanying editorial)

**SUBMITTED**

Chacko AD, Barbone D, Crawford N, Johnston PG, Cilli M, Piccardi F, Bertino P, Vialet J, Mutti L, BROADDUS VC, Gaudino G, Fennell DA. Reversal of bortezomib resistance in mesothelioma by a BH3 peptidomimetic targeting MCL-1/A1. (Submitted)

**EDITORIALS**

1. BROADDUS VC, Light RW. What is the origin of pleural transudates and exudates? Chest 102:658-659, 1992.

2. BROADDUS VC. Asbestos, the mesothelial cell and malignancy: a matter of life or death. Am J Respir Cell Mol Biol 17:657-659, 1997.

3. BROADDUS VC. Apoptosis and asbestos-induced disease – is there a connection? J Lab Clin Med 137(5):314-5, 2001.

4. BROADDUS VC. Diuresis and transudative effusions-changing the rules of the game. Am J Med 110(9):732-5, 2001.

**WORKSHOP OR MEETING SUMMARIES**

1. Crapo JD, BROADDUS VC, Brody AR, Malindzak G, Samet J, Wright JR; American Thoracic Society. ATS-NIEHS Workshop on lung disease and the environment; Where do we go from here? Am J Respir Crit Care Med 168(2):250-4, 2003.

2. BROADDUS VC. Advances in Mesothelioma Research. Report of the IMIG meeting. International Pleural Newsletter 2 (3): 10-11, 2004.

3. Carbone M, Albelda SM, BROADDUS VC, Flores RM, Hillerdal G, Jaurand, M-C, Kjaerheim K, Pass HI, Robinson B, Tsao A. Meeting Review: 8th International Mesothelioma Interest Group Oncogene 26 (49): 6959-6967, 2007.

4. Boylan A, Broaddus VC. Pleural Disease. In: Respiratory Disease in America: An ATS Fact Book. Schraufnagel, DE Editor 2010..

**REVIEWS**

1. Staub NC, BROADDUS VC, Zylak C, Lai-Fook SJ, Light RW, Vinegar R, Gaensler EA, Sahn SA. Pathophysiology of the pleural space. Am Rev Respir Dis 134:820-821, 1986.

2. BROADDUS VC, Berthiaume Y, Biondi JW, Matthay MA. Hemodynamic management of the adult respiratory distress syndrome. J Intensive Care Med 2:190-213, 1987.

3. BROADDUS C and Staub NC. Pleural liquid & protein turnover in health & disease. Sem in Respir Med 9:7-12, 1987.

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5. Matthay MA, BROADDUS VC. Fluid and hemodynamic management in acute lung injury. Sem in Respir Med 15:271-288, 1994.

6. BROADDUS, V.C. Infections in the pleural space: An update on pathogenesis and management. Sem in Respir Crit Care Med 16:303-314, 1995.

7. Marchi E, BROADDUS VC. Mechanisms of pleural liquid formation in pleural inflammation. Curr Opinion in Pulmonary Med 3:305-309, 1997.

8. Nishimura SL, BROADDUS VC. Asbestos-induced pleural disease. Clinics in Chest Medicine 19 (2): 311-329, 1998.

9. Leard LE, BROADDUS VC. Mesothelial cell proliferation and apoptosis. Respirology 9: 292-299, 2004.

10. Mutti L, BROADDUS VC. Malignant mesothelioma as both a challenge and an opportunity. Oncogene 23:9155-9161, 2004.

**CHAPTERS**

1. BROADDUS VC, Wiener-Kronish JP. Pleural diseases. In: Annual Review of Pulmonary and Critical Care Medicine, 1986-1987. Philadelphia: Hanley & Belfus, 1986, 115-132.

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3. Wiener-Kronish JP, BROADDUS VC. Pleural diseases. In: Annual Review of Pulmonary and Critical Care Medicine, 1988-1989. Philadelphia: Hanley & Belfus, 1989, 85-102.

4. Wiener-Kronish JP, Boylan AM, BROADDUS VC. Pleural diseases. In: Annual Review of Pulmonary and Critical Care Medicine, 1991-1992. Philadelphia: Hanley & Belfus, 1991, 93-106.

5. BROADDUS VC. Cardiac diseases. In: Pulmonary Manifestations of Systemic Disease. JF Murray, ed. New York: Marcel Dekker, Inc., 1991; 59:149-190.

6. Light RW, BROADDUS VC. Disorders of the pleura: general principles and diagnostic approach. In: Textbook of Respiratory Medicine. JF Murray, JA Nadel, eds. 2nd edition. Philadelphia: WB Saunders Co., 1994, 2145-2163.

7. BROADDUS VC. Mechanisms of Pleural Liquid Turnover in the Normal State. UptoDate in Pulmonary and Critical Care Medicine (CDROM), S.E. Weinberger, Editor, American Thoracic Society, 1996-2004.

8. BROADDUS VC. Mechanisms of Pleural Liquid Accumulation in Disease. UptoDate in Pulmonary and Critical Care Medicine (CDROM), S.E. Weinberger, Editor, American Thoracic Society, 1996-2004.

9. BROADDUS VC, Hébert, CA. The Role of IL-8 in Inflammatory Diseases. In: Chemoattractant Ligands and Their Receptors. R. Horuk, Editor. CRC Press: New York. 1996, pp. 1-28.

10. Bigelow JM, BROADDUS VC. Empyema and Lung Abscess. In: Pulmonary/Respiratory Therapy Secrets. P.E. Parsons, J.E. Heffner, Editors. Hanley & Belfus, Inc. Medical Publishers, Phila, PA. 1996, pp. 179-185.

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12. BROADDUS VC, Light RW. Disorders of the Pleura: General Principles and Diagnostic Approach (Chapter 73). In: Textbook of Respiratory Medicine. JF Murray, JA Nadel, RJ Mason, HA Boushey, eds. 3rd edition. Philadelphia: WB Saunders Co., 2000; pp. 1995-2012.

13. Light RW, BROADDUS VC. Pleural Effusion. (Chapter 74) In: Textbook of Respiratory Medicine. JF Murray, JA Nadel, RJ Mason, HA Boushey, eds. 3rd edition. Philadelphia: WB Saunders Co., 2000; pp. 2013-2041.

14. Light RW, BROADDUS VC. Pneumothorax, Chylothorax, Hemothorax and Fibrothorax. (Chapter 75) In: Textbook of Respiratory Medicine. JF Murray, JA Nadel, RJ Mason, HA Boushey, eds. 3rd edition. Philadelphia: WB Saunders Co., 2000; pp. 2043-2066.

15. Light RW, BROADDUS VC. Tumors of the Pleura. (Chapter 76) In: Textbook of Respiratory Medicine. JF Murray, JA Nadel, RJ Mason, HA Boushey, eds. 3rd edition. Philadelphia: WB Saunders Co., 2000; pp. 2067-2078.

16. BROADDUS VC, Jaurand MC. Asbestos Fibers and the Biology of Mesothelial Cells. In: Mesothelioma. P Chahinian, BWS Robinson, ed. Gordon & Breach Science Publishers, Harwood Academic Publishers 2004.

17. Bigelow JM, BROADDUS VC. Empyema and Lung Abscess. In: Pulmonary/Respiratory Therapy Secrets. 2nd edition. P.E. Parsons, J.E. Heffner, Editors. Hanley & Belfus, Inc. Medical Publishers, Phila, PA. 2001.

18. BROADDUS VC. Transudative pleural effusions. In: Pleural Diseases. Loddenkemper R, Antony V, Editors. Eur Respir Monograph 2002: 22, pp. 157-176.

19. Nahid P, BROADDUS VC. Liquid and Protein Exchange. (Chapter 3) In: Pleural Diseases: an International Textbook. Light RW and Lee G.Y.C. Editors Hodder Arnold, London, England 2003; pp. 35-44.

20. BROADDUS VC. Physiology. (Chapter 6) and Transudates (Chapter 27). In: Derrame Pleural (Translation: Pleural Effusion). Vargas FS, Teixeira LR, and Marchi E, Editors. Roca Publishers, Brazil, 2003; pp. 15-24 and 233-248.

21. BROADDUS VC, Light RW. Pleural effusion (Chapter 68). In: Textbook of Respiratory Disease. RJ Mason, VC Broaddus, JF Murray, JA Nadel, eds. 4th edition. Philadelphia: Elsevier 2005; pp.1913-1960.

22. Boylan AM, BROADDUS VC. Tumors of the pleura (Chapter 70). In: Textbook of Respiratory Disease. RJ Mason, VC Broaddus, JF Murray, JA Nadel, eds. 4th edition. Philadelphia: Elsevier 2005; pp. 1989-2009.

23. BROADDUS VC. Fluid and solute exchange in normal physiological states. In: Textbook of Pleural Diseases, 2nd Edition. Light RW, Lee YGC (eds) London: Hodder, Arnold, 2008:43-48.

24. Light RW, BROADDUS VC. Pleural Effusion. In: Murray Nadel Textbook of Respiratory Medicine. RJ Mason, VC Broaddus, JF Murray, JA Nadel, King TE, Martin T, Schraufnagel D, eds. 5th edition. Philadelphia: Elsevier., 2010 (in press).

25. BROADDUS VC, Robinson BWS. Pleural Tumors. In: Murray Nadel Textbook of Respiratory Medicine. RJ Mason, VC Broaddus, JF Murray, JA Nadel, King TE, Martin T, Schraufnagel DE, eds. 5th edition. Philadelphia: Elsevier, 2010 (in press).

## RESEARCH PROGRAM
## SIGNIFICANT PUBLICATIONS

BROADDUS VC, Dansen TB, Abayasiriwardana KS, Wilson SM, Finch AF, Swigart LB, Hunt AE, Evan GI. Bid mediates apoptotic synergy between TNF-related apoptosis-inducing ligand (TRAIL) and DNA damage. J Biol Chem 2005; 280:12486-12493.

*We uncovered the molecular mechanism of apoptotic synergy between the damage-intrinsic pathway of apoptosis and the death receptor-extrinsic pathway, showing that the DNA damage pathway sensitized the mitochondria to the Bid signal generated by TRAIL. (I performed all of the experiments during my sabbatical in Gerard Evan's laboratory, directed the work under his guidance and wrote most of the paper. This work has led to ongoing collaboration and follow up studies).*

For all the remaining publications performed in my own laboratory after my sabbatical, I had the original idea, directed all experiments, and wrote most of the paper.

Kim KU, Wilson SM, Abayasiriwardana K, Collins R, Fjellbirkeland L, Xu Z, Jablons DM, Nishimura SL, BROADDUS VC. A novel in vitro model of human mesothelioma for studying tumor biology and apoptotic resistance. Am J Respir Cell Mol Biol 2005; 33(6):541-8.

*We developed a new ex vivo model designed to explore the apoptotic responses of actual human tumor. A role for the Akt/mTOR pathway was found in the apoptotic resistance of the human tumors. This has led to further collaborations and other papers submitted and in preparation.*

Wilson SM, Barbone D, Yang TM, Jablons DM, Bueno R, Sugarbaker DJ, Nishimura S, Gordon GJ, BROADDUS VC. mTOR mediates survival signals in malignant mesothelioma grown as tumor fragment spheroids. Am J Respir Cell Mol Biol 39(5):576-583, 2008.

*We advanced the human tumor ex vivo model first described in our previous study to study a particular survival pathway, Akt/mTOR, and showed that blockade of this pathway did sensitize certain tumors, but only those with high baseline activity of mTOR pathway. Such studies explain how biomarkers of mTOR activity could select patients for therapy with mTOR blockers.*

Barbone D, Yang TM, Morgan JR, Gaudino G, BROADDUS VC. Mammalian target of rapamycin contributes to the acquired apoptotic resistance of human mesothelioma multicellular spheroids. J Biol Chem 283(19):13021-13030, 2008.

*We developed an in vitro 3D model of tumor cells that demonstrated acquired multicellular resistance to apoptosis, and we uncovered one pathway that contributed to resistance, the mTOR pathway. Such studies have informed use of mTOR inhibitors in clinical trials.*

Yang TM, Barbone D, Fennell DA, BROADDUS VC. Bcl-2 family proteins contribute to apoptotic resistance in lung cancer multicellular spheroids. Am J Respir Cell Mol Biol 2009 (Epub ahead of print) * ***With accompanying editorial.***

*We developed 3D spheroids in lung cancer and showed for the first time a role for the Bcl-2 family of proteins in the acquired multicellular apoptotic resistance to bortezomib, the proteasome inhibitor that currently has little activity in solid tumors. This points the way to the use of small molecule Bcl-2 inhibitors to enhance efficacy of bortezomib in lung cancer clinical trials.*

## RESEARCH PROGRAM
### DESCRIPTION OF CURRENT RESEARCH

My research program continues to focus on the apoptotic resistance of tumors, malignant mesothelioma and lung cancer. We have focused particularly on mesothelioma, as a highly refractory and chemoresistant tumor, and are applying our findings now to lung cancer. We are investigating the apoptotic signaling involved in bypassing resistance and inducing apoptosis, specifically by combining agonists of the two major apoptotic pathways: the death receptor pathway and the DNA damage/mitochondrial pathway. We have shown that, while each pathway alone fails to induce apoptosis in the tumor cells, the combination will induce synergistic apoptosis. We continue to explore this phenomenon, searching for non-toxic means of stimulating these pathways such as heat stress and JNK stimulators such as anisomycin.

We have incorporated 3-dimensional models in our study of resistance, both of the mesothelioma cell lines grown as multicellular spheroids and of the human mesothelioma tumor itself grown as tumor fragment spheroids. Both these models allow us to test the resistance in a more clinically relevant system. We have found that a major survival pathway, the PI3K/Akt/mTOR pathway, contributes to the resistance in these 3-dimensional structures. Ultimately however, we believe that the resistance is manifested at the mitochondria by an altered repertoire of anti- and pro-apoptotic molecules. In an ongoing collaboration with Dr. Dean Fennell of Belfast, No. Ireland, we are now exploring the mitochondria as a central integrator of apoptotic signaling.

As a further direction using 3-dimensional models, we are collaborating with Dr. Lisa Coussens in studying the interaction of tumor-associated macrophages with the tumor cells. We will use our models, 3D multicellular spheroids and human tumor fragments, to investigate the contribution of macrophages to the apoptotic resistance of the tumor cells. We have found that mesothelioma contains a high number of macrophages, far more than in lung cancer or other tumors; thus, if these macrophages can be eliminated or manipulated to change from a supportive role to an anti-tumor role, this could be of significant therapeutic benefit for this currently incurable tumor.

**CURRICULUM VITAE**
June 2010

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## I. EDUCATION:

| | | | |
|---|---|---|---|
| 1976 - 1980 | San Francisco State University | B.A. | Biology |
| 1988 - 1993 | University of California, Los Angeles | Ph.D. | Biological Chemistry |
| 1993 - 1997 | University of California, San Francisco | Post-Doctoral Fellow | Cancer Biology |

## II. PRINCIPAL POSITIONS HELD:

| | | | |
|---|---|---|---|
| 1981 - 1988 | Genentech, Inc., South San Francisco | Research Associate | Molec. & Devel. Biology |
| 1997 - 1999 | Univ. of California, San Francisco | Assistant Research Biochemist | Hormone Research Inst. |
| 1999 - 2004 | Univ. of California, San Francisco | Assistant Professor, In Residence | Cancer Research Inst. & Dept of Pathology |
| 2004 - 2006 | Univ. of California, San Francisco | Associate Professor, In Residence | Cancer Research Inst. & Dept. of Pathology |
| 2006 - 2007 | Univ. of California, San Francisco | Associate Professor | Dept. of Pathology & Cancer Research Inst. |
| 2007 - present | Univ. of California, San Francisco | Professor | Dept. of Pathology & Cancer Research Inst. |

**OTHER POSITIONS HELD CONCURRENTLY:**

| | | | |
|---|---|---|---|
| 1989 - 1992 | Whittier College, Whittier, CA | Lecturer | Biology Dept |
| 1992 | Genentech, Inc., South San Francisco | Scientific Consultant | Dept. of Legal Affairs |
| 2000-present | Helen Diller Family Comprehensive Cancer Center, UCSF | Co-Director | Mouse Pathology Core |
| 2007-2009 | *CANCER RESEARCH* | Deputy Editor | |
| 2009-present | Helen Diller Family Comprehensive Cancer Center, UCSF | Co-Director | Program in *Cancer Immunity & Microenvironment* |
| 2009-present | *CANCER RESEARCH* | Deputy Editor | *Breaking Advances* section |

## III. HONORS AND AWARDS:

| | | |
|---|---|---|
| 1985 | Recognition Award | Genentech, Inc., |
| 1986 | Recognition Award | Genentech, Inc., |
| 1988 | Recognition Award | Genentech, Inc. |
| 2000 - 02 | Hellman Family Award For Early Career Faculty | Univ. of Calif., San Francisco |
| 2000 - 01 | V Foundation Scholar | The V Fndt. for Cancer Research |
| 2000 - 03 | Edward Mallinckrodt, Jr. Fndt. Award for Medical Research | Edward Mallinckrodt, Jr. Fndt. |
| 2002 | Gertrude B. Elion Cancer Research Award | Am. Assoc. for Cancer Research |
| 2006 - 11 | Era of Hope Scholar Award | Department of Defense, Breast Cancer Research Program |

## IV. PROFESSIONAL ACTIVITIES

| | | | |
|---|---|---|---|
| 1999 - present | Member | Graduate Program in BioMedical Sciences (BMS) | Univ. of Calif., San Francisco |
| 1999 - present | Member | Helen Diller Family Comprehensive Cancer Center | Univ. of Calif., San Francisco |
| 2000 - present | Member | Graduate Program in Biological Sciences (PIBS) | Univ. of Calif., San Francisco |
| 2001 - present | Co-Director | Mouse Pathology Core | Helen Diller Family Comprehensive Cancer Center, Univ. of Calif., San Francisco |
| 2004 – present | Member | Program in Immunology | Univ. of Calif., San Francisco |
| 2004 – 2007 | Senior Editor | ***Cancer Research (****Cell, Tumor and Stem Cell Biology* Section) | American Association Cancer Research |
| 2007 – 2009 | Deputy Editor | ***Cancer Research (****general****)*** | American Association Cancer Research |
| 2007 – 2009 | Senior Editor | ***Cancer Research (****Tumor Microenvironment* Section) | American Association Cancer Research |
| 2007 - 2010 | Member | External Scientific Advisory Board, University of Minnesota Cancer Center | University of Minnesota Cancer Center |
| 2007 – 2011 | Member | External Scientific Advisory Board, CA U54: *Aging, Tumor Microenvironment and Prostate Cancer*; P.I. Steve Plymate, Univ. of Washington, HMC. | University of Washington |
| 2008 – 2011 | Member | Board of Directors (*elected*) | American Association of Cancer Research |
| 2009 - present | Member | External Scientific Advisory Board; Neuroblastoma Program Project Grant | Children's Hospital Los Angeles, Univ. of Southern California |
| 2009 – 2011 | Deputy Editor | ***Cancer Research (****Breaking* | American Association |

| | | | |
|---|---|---|---|
| | | *Advances section*) | Cancer Research |
| 2009 - present | Co-Director | Program in *Cancer Immunity and Microenvironment* | Helen Diller Family Comprehensive Cancer Center, Univ. of Calif., San Francisco |
| 2010-2012 | Council Member | *Women in Cancer Research*, Council (elected) | American Association of Cancer Research |
| 2010 | Member | External Advisory Committee, P01, Program in *Motility and Invasion*, John Condeelis, Ph.D., PI, Program Director | Albert Einstein College of Medicine |

**Local, National and International Meetings Organized:**

2005 Keystone Symposia, *Inflammation and Cancer*, Co-organizer with Dr. Ray DuBois, Vanderbilt Univ, TN), Breckinridge, CO, USA

2006 5th Annual Timberline Symp. on Epithelial Cell Biology, '*Intrinsic and Microenvironmental Regulation of Epithelial Cancer',* Co-Organizer with Dr. Harold Moses (Vanderbilt University, TN, USA), Timberline, OR, USA

2006 Co-Organizer (with Dr. Lewis Lanier), UCSF HDFCCC Annual Symposium, *'Inflammation & Cancer: Bench to Bedside'.*

2007 Keystone Symposia, *Inflammation and Cancer*, Co-Organizer with Drs. Fran Balkwill (Cancer Research UK) and Glenn Dranoff (Beth Israel Cancer Center, Harvard, MA); Santa Fe, New Mexico, USA

2008 AACR Special Conference: *Inflammation and Cancer*, Co-organizer with Drs. Michael Karin and Larry Marnett. Oahu, Hawaii, USA.

2008 International Society for Biological Therapy of Cancer (ISBTc), *2008 Workshop on Inflammation in Cancer Development,* Co-Organizer with Drs. Michael Karin, (UCSD), Steven Dubinett (UCLA), and George Weiner (WU); San Diego CA USA

2010 Co-Organizer (with Dr. Lewis Lanier), UCSF HDFCCC Program in *Cancer Immunity and Microenvironment* Symposium

2011 AACR Special *Conference: Tumor Microenvironments: New Therapeutic Opportunities*, Co-Organizer with Drs. Kornelia Polyak and Melody Swartz.

## V. PROFESSIONAL ORGANIZATIONS

**Memberships**

| | |
|---|---|
| 2000 - 2009 | American Society for Matrix Biology |
| 2000 - present | American Association for Cancer Research |
| 2001 – 2008 | American Society for Cell Biology |
| 2004 - present | American Society for Investigative Pathology |
| 2004 - 2009 | International Protease Society |

**Service to Professional Organizations**

American Association for Cancer Research

2003 Subsection Co-chair (Tumor Progression, Invasion and Metastasis) Cellular, Molecular and Tumor Biology Subcommittee, AACR Program Committee for *94th Annual Meeting*.

2003 Chair and organizer, Educational Session *(*Proteases: Successes and Failures): *94th Annual Meeting*, Washington D.C., USA

2003 Minisymposium Co-chair (Inflammatory Mediators & Cancer): *94th Annual Meeting*, Washington D.C., USA
2004 - 2006 Member, Grants Committee
2005 Minisymposium Co-Chair (Inflammation, Microenvironment and Tumor Progression): *96th Annual Meeting*, Anaheim, CA USA
2005 Session Chair (Inflammation*)*: *AACR Special Conference: Cancer, Proteases and the Microenvironment*, Bonita Springs, Florida. USA
2006 Subsection Co-chair (Tumor Progression, Invasion and Metastasis) of the Tumor Biology Subcommittee, AACR Program Committee for *97th* Annual meeting
2006 Minisymposium Co-Chair (Inflammation and Cancer): *97th Annual Meeting*, Washington DC, USA
2006 Co-Chairperson, Program Committee: *6th Annual Frontiers in Cancer Prevention Research Conference*, December 5-8, 2007, Philadelphia, PA USA.
2006-2009 Steering Committee Member: AACR Tumor Microenvironment Working Group (TME/AACR).
2007 Organizer, Education session (Inflammation and Cancer), *98th Annual Meeting*, Los Angeles, USA
2007 Minisymposium Co-Chair (Tumor Microenvironment): *98th Annual Meeting*, Los Angeles, CA USA
2007 Co-Chairperson, Program Committee: *2008 99th Annual Meeting of the AACR*. April 12-16, 2008, San Diego, CA. USA
2008 Program Committee Member, Tumor Microenvironment Subcommittee for *99th Annual Meeting of the AACR*. April 12-16, 2008, San Diego, CA. USA
2007-2010 Member, AACR Special Conferences Committee
2008 Co-Organizer Special Conference: *Inflammation and Cancer*, with Drs. Michael Karin and Larry Marnett. Oahu, Hawaii, USA.
2008-2011 Member, Board of Directors (elected)
2009 Member, 2009 Education Committee, 2009 100th AACR Annual Meeting, Denver, CO. USA
2009 Organizer and Chair: *Inflammation and Cancer: Novel Mechanisms Regulating Protumor Immunity* Major Symposium, 2009 100th AACR Annual Meeting, Denver, CO. USA
2009 Organizer and Chair: Education Session, *Aspects of the Tumor Microenvironment that Regulate Solid Tumor Development,* 2009 100th AACR Annual Meeting, Denver, CO. USA
2009 Co-Chairperson, Program Committee: *2010 101st Annual Meeting of the AACR*, April 17-21, 2010, Washington, DC USA
2009 Member, Scientific Review Committee for *Stand Up to Cancer Innovative Research Grants*
2009 Member, Selection Committee: *2010 Pezcoller Foundation-AACR International Award for Cancer Research*
2010-2013 Council Member, *Women in Cancer Research* Council (elected)
2010 Co-Chair, Minisymposium '*The Tumor Microenvironment and Therapeutic Strategies" 2010 101st Annual Meeting of the AACR*, April 17-21, 2010, Washington, DC USA

American Society for Cell Biology

2000 American Society for Cell Biology, photo credits in '*Exploring the Cell'* Ed. W. Wells
2001 Table Leader, Career Discussion Lunch, Women in Cell Biology and Education Committee, 40th Annual Meeting, Washington, DC, USA

2001 Co-chair and Co-organizer, Mini-symposium (Microenvironment/Extracellular Matrix in Development and Disease): 40th Annual Meeting, Washington, DC, USA
2003 Table Leader, Career Discussion Lunch, Women in Cell Biology and Education Committee of the ASCB, *42nd Annual Meeting*, San Francisco, CA, USA
2006 Co-Chair Minisymposium (Cancer Mechanisms): *46th Annual Meeting*, San Diego CA, USA

American Cancer Society
1999 14th Annual Excalibur Round Table, San Francisco, CA, USA
2000 San Mateo County Annual Volunteer Meeting, San Mateo, CA, USA

International Society for Preventive Oncology
2002 Session Chair (Chemoprevention): *6th Annual Meeting*, Pasteur Institute, Paris, France.
2002 Poster Judge (Chemoprevention): *6th Annual Meeting*, Pasteur Institute, Paris, France.

International Proteolysis Society
2007 Member, International Scientific Advisory Committee, 5th General Meeting of the International Proteolysis Society, Rion-Patras, GREECE.

International Society for Biological Therapy of Cancer
2008 Co-Organizer, *2008 Workshop on Inflammation in Cancer Development*, San Diego CA, USA

**Service to Professional Publications:**

| | |
|---|---|
| 2003 - 2005 | Associate Editor, ***Cancer Research*** |
| 2005 – 2007 | Editorial Board, ***Carcinogenesis*** |
| 2004 – 2007 | Senior Editor, ***Cancer Research (****Cell, Tumor and Stem Cell Biology* Section) |
| 2007 – 2009 | Senior Editor, ***Cancer Research (****Tumor Microenvironment* Section) |
| 2007 – 2009 | Deputy Editor, ***Cancer Research*** |
| 2007 | Guest Editor, PNAS Editorial Board |
| 2008 | Guest Editor (with Tyler Jacks), *Current Opinion in Genetics & Development* |
| 2008 – present | Editorial Board, ***Cancer Microenvironment*** |
| 2009 – 2011 | Deputy Editor for Breaking Advances, ***Cancer Research*** |

Ad hoc reviewing
1994 Oncogene;
1995 Am J Pathology; Matrix Biology; J Cell Biology
1999 Am J Pathology; Cancer Letters; Nature Medicine; Nature; PNAS; Cell Motility & the Cytoskeleton; Cancer Research
2000 Am J Pathology; Cancer Research; Genes & Development; Int. J Cancer
2001 J Cell Biology; Int. J of Cancer; EMBO; Neoplasia; Cancer Research
2002 Cancer Research; Am J Pathology; Int. J Cancer; Biological Chemistry; Cancer Cell; Cancer Letters
2003 PNAS; Cancer Research; Int. J of Cancer; J Molecular Medicine; Biological Chemistry; Science; Cancer Cell; Nature Medicine; J Leukocyte Biology; Neoplasia; Am J Pathology
2004 Lancet; Cancer Cell; Cancer Research; American J Pathology; J Cell Biology; Nature Reviews Immunology; Nature Reviews Cancer; PNAS; J Biological Chemistry; Nature; J Exp Med; Int J Cancer
2005 Nature Medicine, Cancer Cell, Cancer Research; Am J Pathology; Cell; Nature; Nature Reviews Immunology; Nature Reviews Cancer; Carcinogenesis
2006 Nature Reviews Cancer; Nature; Nature Medicine; Cell; Cancer Research; Clinical Cancer

Research; J Exp Med; Cancer Cell: Am J Pathology; J Cell Biology
2007 Cell; Nature; PNAS: J Cell Biology; Cancer Research; J Exp Med; Breast Cancer Research
2008 Cancer Cell; PNAS; J Immunology; Nature; J Exp Med; Trends in Genetics; Current Opinions in Investigational Drugs
2009 Cancer Cell; Cell; Nature; J Exp Med; J Clin Invest, Cancer Research; Int J Cancer; Oncogene, J Immunology
2010 Nature, J Exp Med, Nature Medicine, J Invest Dermatology, Cell, Cancer Cell, J Clinical Onc; PNAS; J Clin Invest; Cancer Research; Dis Mech Models;

## VI. INVITED PRESENTATIONS

**Symposia and Workshops**: **International**

1996 *Human Tumor Heterogeneity II*: Cytometric Measurement of Growth Regulation and Genetic Alterations: International Society of Analytical Cytometry. Kananaskas, Alberta, Canada.
1997 *GeneMedicine-Boehringer Mannheim Cancer Alliance: Technology Workshop*. Cancún Mexico.
2001 *2nd Annual International Protease Society*. Freising, Germany.
2002 *6th International Symposium on Predictive Oncology & Intervention Strategies*, Pasteur Institute, Paris, France
2002 **KEYNOTE LECTURE**, *Dutch Cancer Society Annual Symposium*, Luntern, The Netherlands
2002 **KEYNOTE LECTURE**, *Cancer: Genome, Signal & Environment, Takeda Genome Urology International*, Kyoto, Japan
2003 *2nd Annual International Symposium on Epithelial Biology*, Timberline, Oregon USA
2004 10th International Congress of the *Metastasis Research Society*, 'Progress Against Tumor Progression', Genoa Italy
2005 *2005 International Consortium Meeting of the Children's Tumor Foundation: Molecular Biology of NF1, NF2 and Schwannomatosis,* Aspen, CO, USA
2005 *International Symposium on Systems Genome Medicine - Bench to Bedside,* Institute of Medical Sciences University of Tokyo, Tokyo, Japan
2005 *Immunotherapy of Cancer*, XI Annual Symposium of the Danish Cancer Society, Copenhagen, Denmark
2005 *4th General Meeting of the International Proteolysis Society*, Quebec City, Canada
2006 Centro Nacional de Investigaciones Oncológicas (CNIO) Cancer Conference: *Inflammation and Cancer*, Mardid SPAIN
2006 18th Annual Pezcoller Symposium *'Tumor Microenvironment: Heterotypic Interactions'*, Trento ITALY
2006 European Association for Cancer Research (EACR) 1st Annual Meeting, Budapest HUNGARY
2006 XXXIVth Meeting of the International Society for Oncodevelopmental Biology and Medicine (ISOBM: Tumor Biology, Detection and Therapy, Pasadena, CA, USA
2006 37th International Symposium of the Princess Takamatsu Cancer Research Fund '*Cancer Cells and Their Microenvironment*', Tokyo, JAPAN
2007 *4th International Conference on Tumor Microenvironment*, Florence, ITALY
2007 *2nd International Symposium on Cancer Metastasis and the Lymphovascular System*: Basis for Rational Therapy, San Francisco CA USA
2007 CNIO – Nature Symposium on "*Oncogenes and Human Cancer*". The Next 25 Years", Madrid SPAIN
2007 **KEYNOTE LECTURE**, *7th International Symposium on Hodgkin Lymphoma*, Cologne,

GERMANY
2007 **CANDLELIGHT LECTURE**, *Inflammation and Cancer: From molecular links to bed side*; Inaugural meeting for the *Istituto Clinico Humanitas*, Milan ITALY
2008 *7th Annual International Congress on the Future of Breast Cancer*, Kauai, Hawaii USA
2008 Cancer Research UK Cambridge Research Institute (CRI) Inaugural Annual Symposium, '*Unanswered Questions in the Tumour Microenvironment'*, Homerton College, Cambridge UK
2008 5th International Kloster Seeon Meeting, *Angiogenesis: Molecular Mechanisms and Functional Interactions*. Kloster Seeon, GERMANY
2008 **CANCER RESEARCH UK LECTURE**, NCRI Cancer Conference, Birmingham UNITED KINGDOM
2009 21ST Lorne Cancer Conference, Lorne AUSTRALIA
2009 6th International Symposium on the Intraductal Approach to Breast Cancer, Santa Monica CA USA
2009 **STATE-OR-THE-ART LECTURE**, International Cancer Conference, *CANCER 2009*, Dublin IRELAND
2009 19th Annual BioCity Symposium, 'Tumor Microenvironment in Cancer Progression", Tirku FINLAND
2009 **KEYNOTE LECTURE**, European Association of Cancer Research, Special Conference on *Inflammation and Cancer*, Berlin GERMANY
2009 7th International Symposium on Minimal Residual Cancer, Athens, GREECE
2009 Tri-Society Annual Conference of the Society for Leukocyte Biology, International Cytokine Society, and the International Society for Interferon and Cytokine Research, Lisbon, Portugal
2009 5th International Conference on Tumor Microenvironment, Versailles, FRANCE
2009 **PRESIDENT'S PLENARY SESSION**: Italian Cancer Society Annual Meeting, Milano ITALY
2010 **PLENARY LECTURE**, CHUV Research Day, University hospital (CHUV) and the Faculty of Biology and Medicine, Lausanne, SWITZERLAND.

UPCOMING INVITATIONS
2010 *NATURE - CNIO Cancer Symposium* on *Frontiers in Tumour Progression*, Madrid SPAIN
2011 41st Australian Society for Immunology (ASI), Adelaide, South AUSTRALIA

**Symposia and Workshops: National**
1994 *Current Transgenic Technology*, B & K Universal, San Mateo, CA, USA
1997 *Biology of Proteolysis*, Cold Spring Harbor Laboratory, NY, USA
1997 *Molecular Biology & Pathology of Neoplasia*, AACR, Keystone, CO, USA
1997 *Matrix Metalloproteinases*, Gordon Research Conference, Proctor Academy, New London, NH, USA
1998 *Proteolysis*, Gordon Research Conference, Colby-Sawyer College, New London, NH, USA
1998 *Cellular Targets of Viral Carcinogenesis*, AACR Special Conference. Dana Point, CA, USA
1998 *Mechanisms of Tumor Growth & Invasion Mediated by Proteolysis*, UCSF-Molecular Design Institute. San Francisco, CA, USA
1999 *Tumor Microenvironment*, Education Session, AACR Annual Meeting. Philadelphia, PA, USA
1999 *Matrix Metalloproteinases*, Gordon Research Conference, Colby-Sawyer New London, NH, USA.
2000 *Epithelial-Stromal Interactions & Tumor Progression Workshop*, National Cancer Inst., Bethesda, MD, USA
2000 10th National Conference of the Inflammation Research Association, Hot Springs, VA,

USA

2001 *'Meet-the-Expert' Sunrise Session,* AACR Annual Meeting, New Orleans, LA, USA

2002 *Chemotherapy of Experimental & Clinical Cancer*, Gordon Research Conference, Colby Sawyer College, New London, NH, USA

2002 *Proteolytic Enzymes & their Inhibitors*, Gordon Research Conference, Colby Sawyer, New London, NH, USA

2002 *From the Cancer Cell to a Tumor - Tumors as Outlaw Organs,* Schilling Research Conference, The American Cancer Society, Aptos CA, USA

2002 *Cancer Intervention 2002*, Van Andel Research Institute, Grand Rapids, Michigan USA

2002 *Pathobiochemistry B Study Section Workshop,* Natl. Cancer Institute, Hilton Head, SC, USA

2002 *Proteases, Extracellular Matrix and Cancer*, AACR Special Conference, Hilton Head Island, SC, USA

2002 *ECM and Cancer*, Minisymposium, ASCB Annual Meeting, San Francisco, CA, USA

2003 *Matrix Metalloproteinases*, Gordon Research Conference, Big Sky, Montana, USA

2003 *Angiogenesis & Microcirculation*, Gordon Research Conference, Salve Regina, Newport R.I., USA

2003 *Inflammatory Cells and Cancer,* Symposium, American Society of Hematology 2003 Annual Meeting, San Diego, CA, USA

2003 *Validation of a Causal Relationship: Criteria to Establish Etiology*, National Cancer Institute, Cancer Etiology Branch, Washington, DC, USA.

2003 *Functional Imaging of Proteolysis*, Special Session, ASCB Annual Meeting, San Francisco, CA, USA

2004 Scleroderma Research Foundation Annual Scientific Workshop, San Francisco, CA, USA

2004 *Systems Biology of Cancer: The Tumor as an Organ,* Symposium, 95th AACR Annual Meeting. Orlando, FL, USA

2004 *Inflammation and Cancer,* Symposium, 95th AACR Annual Meeting. Orlando, FL, USA

2004 *Remarkable Role of the Microenvironment in Development and Disease Pathogenesis*, Symposium; Experimental Biology 2004, Sponsored by: the Assoc. of Anatomy, Cell Biology and Neurobiology, Washington, D.C., USA.

2004 *Molecular and Cellular Basis of Disease: Structure and Function of the Extracellular Matrix in Disease: Novel Roles and Regulation of MMPs and TIMPs in Disease,* Symposium; Experimental Biology 2004, Sponsored by: the Am. Society of Investigative Pathology, the American Society for Matrix Biology and the North American Vascular Biology organization. Washington, D.C., USA.

2004 Pacific Coast Protease Workshop, Half Moon Bay, CA, USA.

2004 19th Aspen Cancer Conference: *Mechanisms of Toxicity, Carcinogenesis, Cancer Prevention and Cancer Therapy.* Aspen, CO, USA.

2005 Keystone Symposia, *The Role of Microenvironment in Tumor Induction and Progression (J5),* Banff, Alberta CANADA

2005 Keystone Symposia, *Inflammation and Cancer (B8)*, Breckenridge, CO, USA

2005 *Symposium on Inflammation, Repair and Carcinogenesis in Liver, Pancreas and Colon*. UCSF Liver Center and the Program in Gastrointestinal Cancer of the UCSF Cancer Center, Rohnert Park, CA, USA

2005 *In the Forefront of Advances in Cancer Research*, Symposium, 96th AACR Annual Meeting. Anaheim, CA, USA

2005 *Macrophage Symposium*, AMGEN, Seattle, WA, USA

2005 *Immune Response to Cancer Symposium*, 41st Annual Meeting, American Society Clinical Oncology (ASCO), Orlando. FL. USA

2005 *Phagocyte*, Gordon Research Conference, New London, CT, USA

2005 *Mouse Models of Human Cancer Consortium*, Annual Steering Committee Meeting, New Brunswick, NJ USA
2005 *Matrix Metalloproteinases*, Gordon Research Conference, Big Sky, Montana, USA
2005 *Annual Buffalo Regional Conference on Immunology*, Buffalo, NY, USA
2005 2005 Montagna Symposium on *'Tissue repair - molecular mechanisms and clinical challenges'*, Salishan Lodge, OR, USA
2005 4th Annual AACR Conference on *Frontiers in Cancer Prevention Research*, Baltimore MD, USA
2005 AACR Special Conference, *Cancer, Proteases and the Microenvironment*, Bonita Springs, Florida. USA
2006 Timberline Annual Symposium on Epithelial Biology, *Intrinsic and Microenvironmental Regulation of Epithelial Cancer*', Timberline Lodge, Oregon, USA
2006 Keystone Symposium, *Molecular Targets for Cancer Prevention*, Granlibakken Resort, Tahoe City, CA, USA
2006 *Inflammation and Cancer,* Symposium, *97th AACR Annual Meeting*. Washington, D.C., USA
2006 Lineberger Cancer Center's *30th Annual Scientific Symposium*, University of North Carolina, Chapel Hill, North Carolina, USA
2006 KEYNOTE LECTURE, *Vanderbilt-Ingram Cancer Center Retreat 2006*, Vanderbilt University, Nashville TN, USA
2006 **TUMOR BIOLOGY PLENARY LECTURE**, *Advances in Neuroblastoma Research 2006*, Los Angeles, CA, USA
2006 *Genetic, Cellular and Microenvironmental Determinants of Tumor Progression and Metastasis: A 'TPM' Workshop Honoring Martin L Padarathsingh, Ph.D.* TPM Study Section Workshop, Natl. Cancer Institute, Georgetown, VA, USA
2006 ASCO/Federation of European Societies Symposium: *Inflammation in Cancer Progression*, 2006 ASCO Annual Meeting, Atlanta, GA, USA
2006 AACR Special Conference, *Mouse Models of Cancer*, Cambridge, MA, USA
2006 AACR Special Conference, *Tumor Immunology: An Integrated Perspective.* Miami, FL, USA
2007 7th AACR-Japanese Cancer Association Joint Conference: *In the Forefront of Basic and Translational Cancer Research*, Waikoloa, Hawaii, USA
2007 Keystone Symposium, *'Mouse Models at the Frontiers of Cancer Discovery'*, Whistler, British Columbia, CANADA
2007 Keystone Symposium '*Inflammation and Cancer'*, Santa Fe, NM, USA
2007 AAAS Annual Meeting, *Healthy Aging: Inflammation and Chronic Diseases'* Symposium, San Francisco, CA USA
2007 Tumor Microenvironment and Tumor-Stromal Interactions Workshop: Sponsored by Biogen Idec Inc., Oncology Discovery Research, San Diego CA USA
2007 American Thoracic Society 2007 International Conference, *San Francisco Science: Inflammation, Immunity and Signaling*. San Francisco, CA USA
2007 22nd Aspen Cancer Conference: Mechanisms of Toxicity, Carcinogenesis, Cancer Prevention and Cancer Therapy, Aspen CO, USA
2007 Gordon Research Conference, *Epithelial Differentiation & Keratinization*, Bryant University, Smithfield, RI, USA
2007 AACR, *Frontiers in Cancer Prevention Research Conference*, Philadelphia, PA, USA
2007 National Cancer Institute Workshop, '*Profiling of Immune Response to Guide Cancer Diagnosis, Prognosis and Prediction of Therapy'*, Bethesda, MD, USA
2008 47th Midwinter Conference of Immunologists, '*Meeting the challenge: Immunobiology in health and disease*', Asilomar, CA USA

2008 AACR-TREC-NCI Conference on *Energy Balance and Cancer: Mediators and Mechanisms*, Lansdowne, VA USA
2008 Keystone Joint Symposium, '*Cell Death in the Immune System / Cell Death and Cellular Senescence'*, Beaver Run Resort in Breckenridge, CO, USA
2008 Keystone Symposium, '*Inflammation, Microenvironment and Cancer'*, Snowbird Resort in Snowbird, Utah, USA
2008 The John F. Anderson Memorial Lectures in Medicine, '*The Linkage between Inflammation and Cancer*', University of Virginia, Charlottesville VA, USA
2008 *Tumor Microenvironment Symposium*, Stony Brook University, Stony Brook. NY. USA
2008 **KEYNOTE LECTURE**, Fox Chase Cancer Center 13th Annual Postdoctoral Fellow and Graduate Student Symposium, Philadelphia, PA USA
2008 DOD BCRP Era of Hope Meeting 2008, Symposium Session: *Immune and Inflammatory Contributions to Breast Cancer*, AND *Era of Hope Spotlight Session*, Baltimore MD, USA
2008 *AACR Centennial Conference: Translational Cancer Medicine 2008: Cancer Clinical Trials and Personalized Medicine;* Hyatt Regency Monterey in Monterey, CA USA
2008 University of Michigan Comprehensive Cancer Center 2008 Fall Symposium, Ann Arbor MI, USA
2008 *AACR Special Conference*, *Chemical and Biological Aspects of Inflammation and Cancer*, Ko Olina Hawai, USA
2008 International Society for Biological Therapy of Cancer (iSBTc), Workshop on Inflammation in Cancer Development, Westin Horton Plaza San Diego, CA USA
2008 Skirball Symposium, New York University School of Medicine, New York, NY USA
2008 *AACR Special Conference in Cancer Research, Tumor Immunology: New Perspectives*; Miami FL, USA
2009 *1st Conference on Regulatory Myeloid Suppressor Cells*, Clearwater, FL USA
2009 Keystone Symposium, '*Extrinsic Control of Tumor Genesis*, Vancouver, British Columbia CANADA
2009 *Inflammation and Cancer: Novel Aspects of Protumor Immunity,* Major Symposium*,* 100th Annual Meeting AACR, Denver CO USA
2009 2nd Annual Retreat of the CCR-NCI Cancer and Inflammation Program, Gettysburg, PA USA
2009 24th Annual Aspen Cancer Conference, Aspen, CO, USA
2009 2009 Geoffrey Beane Cancer Research Symposium: *Inflammation and Cancer*, Memorial-Sloane Kettering Cancer Center, New York NY USA
2009 AACR Special Conference, Advan*ces in Breast Cancer Research: Genetics, Biology, and Clinical Applications*, San Diego CA USA
2009 NCI's National Tumor Microenvironment Network, Nashville TN USA
2010 Joint Keystone Symposia, *Role of Inflammation in Oncogenesis/Molecular and Cellular Biology of Immune Escape in Cancer*, Keystone CO USA
2010 *3rd Annual Wyeth Discovery Frontiers in Human Disease Symposium*, New York, NY USA
2010 **PLENARY LECTURE**, 2010 *Annual Meeting of the American Association for Cancer Research*, Washington DC USA
2010 *10th Annual Oncology Research Symposium at MIT's Koch Institute for Integrative Cancer Research*. Boston MA USA
2010 *Metastasis and the Tumor Microenvironment*, Short Course, Eppley Institute for Cancer Research, Univ of Nebraska, Omaha, NB USA
2010 *Cancer Cell Biology and Signaling Workshop*, ImClone Systems/Eli Lilly, New York NY, USA

UPCOMING INVITATIONS

2010 Symposium, Center for Excellence in Immunology of the National Cancer Institute, Bethesda MD, USA
2010 *25th Annual Critical Issues in Tumor Microenvironment, Angiogenesis and Metastasis*, Boston MA, USA
2010 Metastasis Research Society-AACR Joint Conference on *Metastasis and the Tumor Micorenvironment, Philadelphia, PA USA*
2010 University of Vermont Cancer Center Symposia, *Inflammation and Cancer*, Burlington VT, USA
2010 **KEYNOTE LECTURE**, *Yves DeClerck Symposium*, Saban Research Institute, University of Southern California and Childrens Hospital Los Angeles, Los Angeles CA, USA
2010 **PLENARY LECTURE**, 2010 American College of Veterinary Pathologists and American Society for Veterinary Clinical Pathology, Concurrent Annuam Meetings, Baltimore MD, USA
2011 2nd International Conference on Immunochemotherapy, entitled "*Immunochemotherapy: Correcting Immune Escape in Cancer*", Philadelphia PA USA
2011 Gordon Research Conference on *Epithelial Differentiation and Keratinization,* Snow Mountain, VT USA
2011 FASEB 2011, Steamboat Grand Resort Steamboat Springs, Colorado USA
2011 Gordon Research Conference on *Tissue Repair and Regeneration*, New Hampshire, USA

**Invited Lectures/Seminars: International**

2000 Medical Genome Center, Division of Molecular Medicine, Australian National University, Canberra, A.C.T. Australia.
2001 German Cancer Center, Heidelberg, Germany.
2001 MERCK Pharmaceutical, Damstedt Germany.
2003 University of Toronto, Ontario Cancer Institute & Princess Margaret Hospital, Toronto, Ontario, CANADA
2004 Cancer Research UK, Barts & The London Queen Mary's School of Medicine & Dentistry, John Vane Science Center, Charterhouse Square, London, UK
2004 Cancer Research UK, London Research Institute, Lincoln's Inn Fields Laboratories, London, UK
2004 University of British Columbia, Department of Biochemistry and Molecular Biology, Vancouver, British Columbia, Canada
2007 Angiogenesis and Tumor Targeting Research Unit & Telethon Institute for Gene Therapy, San Raffaele Scientific Institute, Milan, ITALY
2008 Institute of Cell Biology, ETH Zurich Switzerland
2008 Institute of Cancer and the CR-UK Clinical Centre, Barts & The London School of Medicine and Dentistry, London UK
2009 University of South Hampton, UNITED KINGDOM
2009 The Netherlands Cancer Institute, Amsterdam, THE NETHERLANDS
2010 **DISTINGUISHED GUEST LECTURER**, Institute of Cancer, Barts & London School of Medicine. London GB

UPCOMING INVITATIONS

**Invited Lectures/Seminars: National**

1997 Biologic Therapy Research Conference. Univ. of Pittsburgh Medical Center, Pittsburgh, PA, USA
1997 Immunology Seminar Series. Univ. of Pittsburgh Medical Center, Pittsburgh, PA, USA
1999 Axys Pharmaceuticals, South San Francisco, CA, USA

1999 Berlex Pharmaceuticals, Emeryville, CA, USA
1999 Axys Pharmaceuticals, La Jolla, CA, USA
1999 14th Annual Excalibur Round Table, American Cancer Society, San Francisco, CA, USA
1999 Colloquium in Microbiology, Cell and Molecular Biology. San Francisco State Univ., San Francisco, CA, USA
2000 Chiron Corporation, Emeryville, CA, USA
2000 Oral and Pharyngeal Cancer Branch/NIDCR, National Institutes of Health, Bethesda, MD, USA
2000 Fibrogen, Inc., South San Francisco, CA, USA
2000 Scios Inc., Sunnyvale, CA, USA
2000 Molecular Biology Department, University of Southern California, Los Angeles, CA, USA
2001 Dept. of Pediatric Hematology and Oncology, Children's Hospital Los Angeles, Univ. of Southern California, Los Angeles, CA, USA
2001 Jonnson Comprehensive Cancer Center, Univ. of Calif., Los Angeles, Los Angeles, CA, USA
2002 Institute for Engineering and Medicine, Univ. of Pennsylvania, Philadelphia, PA, USA
2002 Oncology Grand Rounds, Univ. of Missouri, Columbia, MO.
2002 Cancer Center, Univ. of California, Davis, Davis CA, USA
2002 AstraZeneca, Waltham, MA USA
2002 Pharmacology Seminar Series, Dept. of Pharmacology, Wayne State Univ., Detroit, MI, USA
2003 Dept. of Biology, Univ. of Calif., San Diego, San Diego, CA USA
2003 Tularik, Inc., South San Francisco, CA USA
2003 Dept. of Cancer Biology's Cancer Metastasis Research Program Seminar Series, M.D. Anderson Cancer Center, Univ. of Texas, Houston, TX, USA
2003 Dept. of Cancer Biology, Stanford University, Stanford, CA, USA
2004 Burnham Cancer Institute, San Diego, CA, USA
2004 The Wistar Cancer Institute, Philadelphia, PA, USA
2004 Regeneron Pharmaceuticals, Inc. Tarrytown, New York, USA
2004 Keynote Lecture: Vanderbilt University Digestive Disease Research Center Retreat, Vanderbilt University, Nashville, TN, USA
2004 Dana Farber Cancer Center, Harvard Medical School, Boston MA, USA
2004 Indiana University, Herman B. Wells Center for Pediatric Research and Clinical Cancer Center, Indianapolis IN, USA
2004 Immunology Graduate Program Seminar, Stanford University, Stanford, CA, USA
2005 Dept. of Nutritional Sciences & Toxicology, Univ. of Calif., Berkeley, Berkeley, CA USA
2005 Rigel, Inc., South San Francisco, CA USA
2005 Dept of Pathology & Lab Medicine, Univ. of California, Los Angeles, Los Angeles, CA USA
2006 Division of Cancer Biology and Angiogenesis in the Department of Pathology at Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA USA
2006 Department of Molecular and Medical Pharmacology, University of California, Los Angeles, Los Angeles, CA USA
2007 *Lymphoma and Myeloma Conference*, M.D. Anderson Cancer Center, Houston, TX, USA
2007 University of Minnesota, Dept. of Lab Medicine and Pathology, Minneapolis, MN, USA
2007 Memorial-Sloan Kettering Cancer Center, Program in Cancer Biology and Aging, New York NY, USA
2007 Abramson Family Cancer Research Institute and Univ. of Pennsylvania, Division of Hematology-Oncology, Philadelphia, PA USA
2007 Albert Einstein College of Medicine, New York NY, USA

2007 Oncology Division Research, Biogen Idec Inc., San Diego, CA USA
2007 Genentech, Inc. Immunology Program. South San Francisco, CA USA
2007 University of Iowa Carver College of Medicine, Dept of Pathology, *Pathology Grand Rounds*, Iowa City, Iowa, USA
2007 Hollings Cancer Center, Medical University of South Carolina, Charleston, SC, USA
2007 University of Michigan, Program in Immunology and Cancer Research Series, Ann Arbor, MI USA
2008 Department of Pathology/UCLA School of Medicine Seminar, Los Angeles CA USA
2008 ANNUAL KEYNOTE LECTURE, Dept of Cancer Biology, Meharry Medical College, Nashville, TN USA
2008 University of California, Davis Cancer Center, Sacramento, CA USA
2008 Department of Immunology, University of Pittsburgh School of Medicine. Pittsburgh, PA, USA
2008 Cancer Biology Series, Ben May Cancer Center, University of Chicago, Chicago, IL, USA
2008 National Cancer Institute Center for Cancer Research Grand Rounds Series in Clinical and Molecular Oncology. Bethesda MD, USA
2009 University of Michigan, Oral Health Sciences Program and Biomedical Engineering Seminar Series, Ann Arbor, MI USA
2009 Department of Pharmacology, Wayne State University, Detroit, MI USA
2009 Molecular Biology Seminar Series, Biochemistry and Molecular Genetics, University of Colorado Health Sciences Center, Aurora, CO USA
2009 National Institutes of Health/National Cancer Institute, Vascular Biology Seminar Series, Bethesda MD, USA
2009 Genentech, Inc., Molecular Oncology Program. South San Francisco, CA USA
2009 Breast Cancer Network of Strength, California Breast Cancer Organizations, Northern California Affiliate, David CA USA
2009 Fred Hutchinson Cancer Center, Seattle WA USA
2010 Cold Spring Harbor Laboratory, CSH NY USA
2010 Albert Einstein College of Medicine, New York, NY USA
2010 Department of Cell Biology & Physiology Washington University, St Louis, MO USA
2010 Cancer Center Seminar Series at Burnham Institute for Medical Research, San Diego CA, USA
2010 Oncology Seminar Series, MedImmune, Gaithersburg, MD, USA

UPCOMING INVITATIONS
2010 Immunology Institute Seminar Series, Mt Sinai School of Medicine, NY, NY USA
2011 Hematology & Medical Oncology, Tulane Univ. School of Medicine, New Orleans, LA USA
2011 McArdle Seminar in Cancer Biology series, Univ of Wisconsin-Madison, USA
2011 Duke University Medical Center, Durham, North Carolina USA
2011 Tulane Cancer Center, Tulane University, New Orleans LA, USA
2011 Seminars in Oncology Lecture Series, Dana-Farber Cancer Institute and the Dana-Farber/Harvard Cancer Center, Boston MA, USA

**Invited Lectures/Seminars: UCSF**
1997 Breast Cancer SPORE Seminar. UCSF
1999 Cancer Research Institute Retreat, Tomales Bay, CA
2000 Chemistry and Cancer: How Chemistry-Based Tools Are Helping Solve Today's Serious Health Problems, Dev. & Alumni Relations, UCSF
2000 Oncology Grand Rounds, Department of Hematology and Oncology, UCSF

2000 PIBS-Cell Biology Seminar Series, UCSF
2000 Pathology and Lab Medicine Grand Rounds, UCSF
2000 BMS Student Pizza Talk, UCSF
2000 Cell Cycle & Dysregulation Club, Comprehensive Cancer Center, UCSF
2000 Comprehensive Cancer Center Retreat, Granlibakken, Tahoe City, CA
2001 BMS Student Pizza Talk, UCSF
2001 Pathology and Lab Medicine Grand Rounds, Departments of Medicine and Pathology, UCSF
2001 UCSF, Cell Biology Retreat, Wilbur Hot Springs, CA, USA
2001 UCSF TETRAD Retreat, Granlibakken, Lake Tahoe, CA, USA
2001 UCSF Cancer Research Institute/BMS Retreat, Granlibakken, Lake Tahoe, CA. USA
2002 Current Topics in Medical Science, UCSF Medical Scientist Training Program (M170.09)
2002 Mouse Models of Human Cancer Program, Comprehensive Cancer Center, UCSF
2002 Cancer Research Institute Retreat, Santa Cruz, CA
2003 PIBS Student Pizza Talk, UCSF
2003 Breast Oncology Program, Comprehensive Cancer Center, UCSF
2003 Comprehensive Cancer Center Faculty Retreat: *Identification and Functional Assessment of Cancer Effectors*, Golden Gate Club, San Francisco CA
2004 BMS Graduate Program Retreat, Granlibakken Tahoe City, CA
2005 BMS Student Pizza Talk, UCSF
2006 *Introduction to Research,* Department of Pathology, UCSF
2008 Division of Experimental Medicine, Divisional Seminar Series, UCSF
2009 Immunology Program, UCSF
2009 Helen Diller Family Comprehensive Cancer Center *Research Symposium*; UCSF
2010 Bay Area Workshop on Lung Development, Physiology and Cancer, San Francisco CA USA
2010 UCSF-GIVI Center for AIDS Research (CFAR) Scientific Symposium for 2010: *HIV Infection, Inflammation, and Premature Aging*, San Francisco, CA USA
2010 Breast Oncology Program Seminar, Helen Diller Family Comprehensive Cancer Center, UCSF

UPCOMING INVITATIONS
2011 Helen Diller Family Comprehensive Cancer Center Symposium, San Francisco CA, USA

## VII. GOVERNMENT AND OTHER PROFESSIONAL SERVICE:

### GOVERNMENT SERVICE

| | | |
|---|---|---|
| 2003-2006 | National Institutes of Health, Center for Scientific Review | Ad hoc reviewer (10/2003; 02/2005; 10/2005; 06/2006), Tumor Progression & Metastasis (TPM) Study Section, Oncological Sciences Review group |
| 2003 | Division of Cancer Biology, National Cancer Institute: *Microenvironment Think Tank* | Participant and *Reporter* |
| 2003 | Division Cancer Etiology, National Cancer Institute: *Validation of A Causal Relationship: Criteria to Establish Etiology Think Tank* | Invited speaker and Participant |
| 2004 | National Institutes of Health, National Cancer Institute | Subcommittee C (05/2004) – Basic & Preclinical NCI Initial Review Group, |

| | | |
|---|---|---|
| | | NCI-C RPRB (T2) Angiogenesis |
| 2005 | National Institutes of Health, National Cancer Institute | Subcommittee D (02/2005) – Clinical Studies NCI Initial Review Group, NCI-D RPRB Tumor Pathology |
| 2005 | National Institutes of Health, Center for Scientific Review-Oncology | Special Emphasis Panel (SEP); ZRG1 ONC (03) M, Developmental Therapeutics |
| 2010 | National Institutes of Health, Center for Scientific Review-Neuroscience | Special Emphasis Panel (SEP)/Scientific Review Group 2010/05 ZNS1 SRB-R (47) |

**OTHER PROFESSIONAL SERVICE**

| | | |
|---|---|---|
| 1999 | Arkansas Science & Technology Authority | Ad hoc Grant Review |
| 2000 | McGraw-Hill, '*Biology'* 6th edition, Ed. P.H. Raven and G.B. Johnson | Ad hoc Review, Chapters 17 and 18 |
| 2000 | Division of Cancer Biology, NCI: *Epithelial-Stromal Interactions & Tumor Progression Workshop* | Invited speaker and Participant |
| 2001 | Department of Veterans Affairs | Ad hoc Grant Review, Oncology Review Board |
| 2001 | Research Grants Council of Hong Kong | Ad hoc Grant Review |
| 2003 | Danish Cancer Society, DENMARK | Ad hoc Grant Review |
| 2004 | Division of Gastroenterology and Digestive Disease Research Center, Vanderbilt University, Nashville TN, USA | '*H. pylori-induced Inflammation and Gastric Adenocarcinoma*, PO1 External Advisory Panel |
| 2004 | Cancer Research Ireland, Irish Cancer Society | Ad hoc grant review |
| 2004 | Dutch Cancer Society | Ad hoc grant review |
| 2004 | Vanderbilt University, Nashville TN, USA; SPORE in GI Cancer | Ad hoc reviewer for SPORE Developmental Research Program |
| 2005 | Keystone Symposia, *Inflammation and Cancer* | Co-organizer (with Dr. Ray DuBois, Vanderbilt Univ, TN), Breckinridge, CO, USA |
| 2006 | 5th Annual Timberline Symp. on Epithelial Cell Biology, '*Intrinsic and Microenvironmental Regulation of Epithelial Cancer'* | Co-Organizer (with Dr. Harold Moses, Vanderbilt University, TN, USA), Timberline, OR, USA |
| 2006 | Keystone Symposia Cancer Study Group for 2009 programming | Study group member |
| 2007 | Keystone Symposia, *Inflammation and Cancer* | Organizer (with Drs. Fran Balkwill (Cancer Research UK) and Glenn Dranoff (Beth Israel Cancer Center, Harvard, MA) Santa Fe, New Mexico, USA |
| 2008 | AACR Special Conference on '*Inflammation and Cancer'* | Co-Organizer (with Drs. Michael Karin and Larry Marnett) |
| 2007-2010 | University of Minnesota Cancer Center; Douglas Yee, M.D., Director | External Scientific Advisory Board Member |
| 2007 – 2011 | University of Washington, Seattle WA, USA | Member, External Scientific Advisory Board, CA U54 TMEN: *Significance of Microenvironment for Prostate Cancer* |

| | | |
|---|---|---|
| | | *Initiation and Progression*; P.I. Stephen R Plymate, Univ. of Washington School of Medicine. |
| 2007 – 2011 | Albert Einstein College of Medicine of Yeshiva University, New York, NY USA | Member, External Scientific Advisory Board, CA U54 TMEN: *Novel Methods for Detection Cell Interactions in the Tumor Microenvironment*; P.I. John S. Condeelis, Albert Einstein College of Medicine. |
| 2008 | International Society for Biological Therapy of Cancer (iSBTc), *2008 Workshop on Inflammation in Cancer Development* | Co-Organizer (with Drs. Michael Karin, Steven Dubinett, George Weiner) |
| 2009 | GlaxoSmith Kline | Member, Tykerb Post-ASCO KOL Advisory Board |
| 2009 | University of Southern California, Children's Hospital | Member, External Scientific Advisory Board, Neuroblastoma, Program Project grant (P01), PI: Robert Seeger, M.D., |
| 2009-2011 | Cancer Prevention and Research Institute of Texas (CPRIT) | Member, Scientific Review Committee; Basic Cancer Biology Review Committee |

## VIII. UNIVERSITY AND PUBLIC SERVICE

### UNIVERSITY SERVICE

System wide

| | |
|---|---|
| 1992-1993 | Graduate Student Representative, Dept. of Biological Chemistry Faculty Council, UCLA |
| 2004 | *ad hoc* Member External Advisory Panel; Jonsson Comprehensive Cancer Center, University of California, Los Angeles, Los Angeles CA, USA |
| 2009 | Member, Site Visit Programmatic Review Group, Department of Pathology & Laboratory Medicine, UCLA School of Medicine. Graduate Council of the UCLA Academic Senate. |

University of California, San Francisco (CAMPUS-WIDE)

| | |
|---|---|
| 1997 | Presentation, Donor Seminar, UCSF Development Office |
| 1998 | Presentation, Donor Seminar, UCSF Development Office |
| 2000 – 2004 | Member, Steering Committee, Ovarian Cancer Program Project Grant |
| 2000 – 2005 | Member, Scholarships and Awards Committee, Academic Senate, School of Medicine |
| 2002 – present | Member, BioMedical Sciences Graduate Program (BMS) Executive Committee |
| 2002 – 2004 | Member, Medical Scientist Training Program Executive Committee |
| 2004 - 2006 | Member, Search Committee, Director of Molecular Imaging, Dept. of Radiology, Committee Chair: Ron Arenson, M.D. no successful recruitment |
| 2004 – 2006 | Member, BioMedical Sciences Graduate Program (BMS); Admissions Committee |
| 2004 | Organizer, BioMedical Sciences Graduate Program Retreat, Granlibakken, N. Lake Tahoe, CA USA |
| 2005 - 2006 | Member, Tissue Engineering Ladder-rank Faculty Search Committee, Dept. of Surgery. Committee Chair: Nancy Boudreau, Ph.D. Successful recruitment of Valerie Weaver, Ph.D. |

2005 - 2009 Member, Ethel and Jane Sokolow Memorial Cancer Endowment Lectureship Committee.

2006 Member, Cancer Faculty Search Committee, Anatomy Dept., Committee Chair: Zena Werb, Ph.D. Successful recruitment of Jeroen Roose, Ph.D.

2006 Member, Faculty Advisory Committee for 2007 Journalist Seminar on *Inflammation and Disease*. Sponsored by Associate Vice Chancellor Barbara J. French

2007 Member, committee to select recipient of Dean's Postdoctoral Prize Lecture.

2007 Member, Faculty Search Committee for Restorative Neurosurgery and Stem Cell Neurobiology, VA Medical Center/UCSF NeuroSurgery. Committee Chair: Linda Noble, Ph.D.; Status: open.

2009 Member, Committee to choose 1st Bonnie J. and Anthony Addario Endowed Chair in Thoracic Oncology, School of Medicine, UCSF

2010 Member, 2010 Selection Committee for the Hellman Family Early-Career Faculty Award.

University of California, San Francisco, Helen Diller Family Comprehensive Cancer Center

1999 Member, Cancer Center Research Building Space Review Policy Committee

1999 – 2002 Member, Mt Zion Animal Barrier Facility Committee

1999 – 2005 Member, Cancer Center Friday Seminar Series Committee

2000 Organizer and Chair, MZ Cancer Center Research Building Annual Retreat

2001 Member, 'Star Performance Award' selection committee

2001 Presentation, Evelyn Herman Reception, UCSF Development Office

2001 – 2002 Member, Cancer Center Research Building, 'Cancer Center Faculty Working Group'

2001 – 2006 Member, Mouse Models of Human Cancer Working Group

2002 – 2003 Member, UCSF Mt Zion campus, Animal Protocol Review Committee

2002 Member, ACS IRG grant review committee

2002 – 2006 Steering Committee Member, Mouse Models of Human Cancer

2003 Member, Review Committee, UCSF Comprehensive Cancer Center Stewart Trust Award

2003 – 2009 Chair, UCSF Mt Zion Campus Animal Protocol Review Committee

2003 Member, Search Committee: Associate Director for Administration, UCSF Comprehensive Cancer Center (Erica Weber, recruited)

2004 Member, Review Committee, UCSF Comprehensive Cancer Center Stewart Trust Award

2006 Co-Organizer, UCSF CCC Annual Symposium, *'Inflammation & Cancer: Bench to Bedside'.*

2008 Chair, Committee to nominate Postdoctoral scholar for AACR 2008 Annual Meeting, Inaugural "Future Leaders, New Directions" Special Symposium. Nominee: Laura Soucek, Ph.D. (awarded)

University of California, San Francisco, Cancer Research Institute

2001 – 2002 Member, Cancer Research Institute Membership Subcommittee

University of California, San Francisco, Department of Pathology

2003 Member, Committee to recommend faculty for the *Robert E. Smith Endowed Chair in Experimental Pathology*

2004 Member, Search Committee, Ladder rank faculty, Physician-Scientist, Anatomic Pathology. Successful recruitment of Jay Debnath, M.D., Ph.D.

2007 Member, Search Committee, Ladder-rank faculty, Physician-Scientist, Pathology

and Neuropathology. Committee Chair: Michael D Prados, M.D.; Status: open.

2008 Member, Search Committee, Ladder-rank faculty, Physician-Scientist, Experimental Pathology. Committee Chair: Benedict Yen, M.D.; Status: open

2009-present Pathology Dept. Academic Merit and Promotions Committee

University (other)

2002 Guest Instructor, Graduate Oncology, University of Missouri, Columbia, Missouri USA

2003 Guest Instructor, Cancer Biology, Stanford University, Stanford, CA USA

2004 Guest Instructor, Immunology, Stanford University, Stanford, CA USA

2008 Guest Instructor, *Exploring the Tumor microenvironment*, Postgraduate course, ISREC, Lausanne University's Biochemistry and Biology Departments, and the Lausanne Branch of the Ludwig Institute, Lausanne Switzerland. Course Organizers, Ivan Stamenkovic and Michel Aguet

**PUBLIC SERVICE:**

1990 Lecturer, Science Academy Of Whittier, Summer Institute. Whittier College, Whittier, CA

1991 Organizer and Lecturer, Science Academy Of Whittier, Summer Institute. Whittier College, Whittier, CA.

1993 Lecturer, Joslyn Community Center. Claremont, CA.

1994 Provided elementary educators with science-related supplies (photos, slides, fixed tissue samples).

1995 Co-Coordinator Hormone Research Institute, 'Take Our Daughters To Work Day', Univ. of Calif., San Francisco

2002 Photo credits and interviewed for '*Misdiagnosis: Failure of Promising Cancer Treatment Starts Soul Searching by Researchers & Drug Companies',* in: *San Francisco Chronicle,* May 12, 2002.

2003 Interviewed for article '*Body's First Defense May Be Root of Diseases',* in: *The Washington Post,* February 20, 2003

2003 Interviewed for article '*The Body on Fire*', in: U.S. News & World Report, October 20, 2003

2004 Interviewed for comments in: *Science News*, '*Early Warming: Inflammatory protein tied to colon cancer risk*" February 7, 2004, Vol 165.

2004 Interviewed for article '*The Fires Within'*, in: *TIME* Magazine, February 23, 2004

2004 Interviewed for comments on AACR Annual Meeting in: *Oncology Times*, '*Exercise Reduces Inflammatory Response, May also Reduce Cancer Risk*', Robert H Carlson, 26(11):33-34, June10, 2004

2004 Interviewed for article *'Inflammation and Cancer: The Link Grows Stronger',* in: *Science*, 306, 966-968 (2004)

2005 Interviewed for article *'Quieting a Body's Defenses',* in: *Newsweek*, Special Edition, Summer 2005

2006 Interviewed for "Expert Commentary" by *BreastLink.org,* on article "Association Between Circulating White Blood Cell Count and Cancer Mortality." *Archives of Internal Medicine*, January 23, 2006; 166:188-194. http://www.breastlink.org/index.php?module=announce&ANN_user_op=view&ANN_id=208

2007 UCSF Research Perspectives 2007 – Inflammation as Cause and Consequences of Disease, Media Event for Journalists, September 27, 2007, UCSF Mission Bay Campus

2007 On-Air radio interview by Dave Iversen, KQED *FORUM*, September 28, 2007 San Francisco CA USA

## IX. TEACHING AND MENTORING

Formal Scheduled Classes for UCSF Students:

| Qtr | Academic Yr | Course No. & Title | Teaching Contribution | Units | Class Size |
|---|---|---|---|---|---|
| W | 1997/98 | IDS 100; Histology Laboratory | *Neoplastic Skin Histopathology*; Laboratory lecture & instruction | 10 | 150 |
| W | 1998/99 | IDS 100; Histology Laboratory | *Neoplastic Skin Histopathology;* Laboratory lecture & instruction | 10 | 150 |
| W | 1999/00 | IDS 100; Histology Laboratory | *Neoplastic Skin Histopathology*; Laboratory lecture & instruction | 10 | 150 |
| S | 1999/00 | BMS 297A; Molecular Biology & Pathology of Neoplasia | *Animal Models of Cancer Laboratory*; Laboratory lecture & instruction | 3 | 15 |
| S | 2000/01 | BMS 297A; Molecular Biology & Pathology of Neoplasia | *Animal Models of Cancer Laboratory*; Laboratory lecture & instruction | 3 | 15 |
| W | 2000/01 | BMS 225; Tissue and Organ Biology | Lecture and laboratory instruction | 3 | 15 |
| S | 2000/01 | BMS 260; Cell Biology | Discussion group leader | 1 | 6 |
| F/W | 2001/02 | IDS 101; Prologue | Laboratory Instructor | 9 | 30 |
| W | 2001/02 | BMS 225; Tissue and Organ Biology | Lecture and laboratory instruction | 3 | 15 |
| W | 2001/02 | IDS 103; Cancer Block | *Invasion & Metastasis*; Lecturer | 7 | 150 |
| S | 2001/02 | BMS 260; Cell Biology | Discussion group leader | 1 | 7 |
| F | 2002/03 | BMS 260; Cell Biology | Discussion group leader | 1 | 6 |
| W | 2002/03 | IDS 103; Cancer Block | *Invasion & Metastasis*; Lecturer | 7 | 150 |
| F/W | 2002/03 | IDS 101; Prologue | Laboratory Instructor | 9 | 30 |
| F | 2003/04 | BMS 260; Cell Biology | Discussion group leader | 1 | 6 |
| S | 2003/04 | BMS 225B, Tissue and Organ Biology | Lecturer and Laboratory Instructor | 1.5 - 5 | tbd |
| W | 2003/04 | Biochem 297; Molecular Biology & Pathology of Neoplasia | *Angiogenesis:* Lecturer | 3 | 30 |
| W | 2003/04 | BMS 297A Molecular Biology & Pathology of Neoplasia Laboratory | Lecturer and Laboratory Instructor, *Animal Models of Neoplasia* | 1 | 10 |
| S | 2003/04 | BMS 225B; Tissue & Organ Biology | Lecturer: Cancer I & Cancer II | 1.5 - 5 | 16 |
| F | 2004/05 | BMS 260; Cell Biology | Discussion group leader | 1 | 6 |
| F | 2005/06 | BMS 260; Cell Biology | Discussion group leader | 1 | 7 |
| W | 2006/07 | Biochem 297; Molecular Biology & Pathology of Neoplasia | *Inflammation and Cancer*: Lecturer | 3 | 30 |
| W | 2008/09 | BMS230; Cellular & Molecular Biology of Cancer | Course Co-Director | 3.5 | 22 |
| W | 2008/09 | BMS230; Cellular & Molecular Biology of Cancer | Lecturer: *Cancer Microenvironments; Inflammation and Cancer* | 3.5 | 22 |
| W | 2010/11 | BMS230; Cellular & Molecular Biology of Cancer | Course Co-Director | 3.5 | 22 |

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|---|---|---|---|---|---|
| W | 2010/11 | BMS230; Cellular & Molecular Biology of Cancer | Lecturer: *Tumor cell heterogeneity*; *Cancer Microenvironments; Inflammation and Cancer* | 3.5 | 22 |

Postgraduate and Other Courses:

| | | |
|---|---|---|
| 1989 | M204, *Biochemistry Lab*<br>Univ. of Calif., Los Angeles | Student Teaching Assistant for quarter long course (100 medical students) |
| 1989 | Biology 250, *Human Heredity*; Dept. of Biology<br>Whittier College, Whittier CA | Organized and taught entire lecture-based course (30 undergraduate students) |
| 1990 | Biology 350 & 350L, *Molecular Genetics*; Dept. of Biology, Whittier College, Whittier CA | Organized and taught entire lecture and laboratory course (16 undergraduate students) |
| 1990 | M204, *Biochemistry Lab*<br>Univ. of Calif., Los Angeles | Student Teaching Assistant for quarter long course (100 medical students) |
| 1990 | Biology 250, *Human Heredity*; Dept. of Biology<br>Whittier College, Whittier CA | Organized and taught entire lecture-based course (30 undergraduate students) |
| 1992 | Biology 350 & 350L, *Molecular Genetics*; Dept. of Biology, Whittier College, Whittier CA | Organized and taught entire lecture and laboratory course (16 undergraduate students) |
| 2003 | Graduate *Oncology*, University of Missouri, Columbia, MS, USA | Invited Guest Lecturer: Lecture syllabus & delivered 2-hr lecture for course (15 students, combination of graduate, medical & postgraduate fellows |
| 2003 | Graduate Program in Cancer Biology, Stanford Univ., Stanford, CA USA | Invited Guest Lecturer: Delivered 1-hr lecture to graduate students in Cancer Biology Graduate program |
| 2004 | Graduate Program in Immunology, Stanford Univ., Stanford, CA USA | Invited Guest Lecturer: Delivered 1-hr lecture to graduate students in Immunology Graduate program |
| 2005 | UCSF Dermatology residents' Basic Science Seminar Series | Invited Guest Lecturer: Delivered 1-hr lecture to UCSF Dermatology Residents (11 M.D. and M.D., Ph.D. Residents) |
| 2008 | ISREC, Lausanne University's Biochemistry and Biology Departments, and the Lausanne Branch of the Ludwig Institute | Guest Instructor: *Exploring the Tumor microenvironment*, postgraduate course. (20 PhD students, 3 hours of instruction) |
| 2009 | OOA Course: Tumor Microenvironment; The Netherlands Cancer Institute | Guest Faculty: (4.5 hours of instruction) |
| 2010 | 25th Annual Harvard Tumor Course: Critical Issues In Tumor Microenvironment, Angiogenesis and Metastasis: *From Bench to Bedside and Back* | Faculty member: (2 hours of instruction) |
| 2010 | Eppley Institute for Research in Cancer, Univ. of Nebraska Medical Center. Short Course in Cancer Biology: Metastasis and the Tumor Microenvironment | Faculty member: (3 hours of instruction) |
| | | |

High School and Undergraduate Students Supervised or Mentored:

| Dates | Name | Program or School | Faculty Role | Current position |
|---|---|---|---|---|
| 1998 | Christopher Tinkle | Undergraduate, Univ. of Texas, Austin, TX, USA | Summer Research Training Program Supervisor | MSTP student, Rockefeller University |
| 2000 | Adam Zucker | Undergraduate, Oberlin College, Ohio USA | Supervised Summer work | unknown |

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|---|---|---|---|---|
| 2000 | Ashkan Hirari | Undergraduate, Univ. of Calif., Berkeley, Berkeley CA, USA | Supervised Summer work | unknown |
| 2001 | Jason Reuter | Undergraduate, Univ. of Calif., Berkeley, Berkeley CA USA | Supervised Summer work | Ph.D. student, Stanford University |
| 2002 | Destinee Cooper | Undergraduate, Univ. of Calif., Davis USA | Summer Research Training Supervisor | unknown |
| 2006 | Sunum Mobin | UCSF Science & Health Education Partnership: High School Intern Program | Summer Research Training Supervisor | unknown |
| 2008-2009 | Julia Lam | Undergraduate, Univ. of Calif., Berkeley, Berkeley CA USA | Independent study (199), Mentor | B.S. U. C. Berkeley 2009 |
| 2010 | Scott Keil | Undergraduate ,The University of Glasgow, Scotland | Summer Research Training Supervisor | The University of Glasgow, Scotland |
| 2010 | Heather Chen | Undergraduate, Univ. of Calif., Berkeley, Berkeley CA USA | Summer Research Training Supervisor | Undergraduate, Univ. of Calif., Berkeley, Berkeley CA USA |
| 2010 | Amy Desalazar | Cupertino High School Cupertino, CA USA | Summer Research Training Supervisor | Cupertino High School |
| 2010 | Nikhil Wadhwani | Undergraduate, Sarah Lawrence College, Bronxville, NY USA | Summer Research Training Supervisor | Sarah Lawrence College, Bronxville, NY |

Predoctoral Students Supervised or Mentored:

| Dates | Name | Program or School | Faculty Role | Current position |
|---|---|---|---|---|
| 2000 | Jin-Sae Rhee | UCSF MSTP/BMS, graduate student | Rotation Supervisor | PhD awarded 2003, M.D. awarded 2005 |
| 2000 - 2003 | Jin-Sae Rhee | UCSF M.D., Ph.D., | Ph.D. supervisor | Pediatric Resident, Children's Hospital, Washington D.C. |
| 2000 | Maria Christophorou | UCSF BMS, graduate student | Faculty coach, BMS 297 | Ph.D. awarded 2006 |
| 2001 | Leslie Chu | UCSF BMS, graduate student | Rotation Supervisor | Ph.D. awarded 2005 |
| 2001 | Rayna Takaki | UCSF BMS, graduate student | Rotation Supervisor | Ph.D. awarded 2006 |
| 2001 – 2002 | Sophia Bruggerman | University of Nijmegan, The Netherlands | Masters Thesis Supervisor | Ph.D. student, The Netherlands Cancer Institute |
| 2002 | Lucy Lebedeva | UCSF PIBS, graduate student | Faculty coach, BMS 297 | Ph.D. awarded 20057 |
| 2002 | Leslie Chu | UCSF BMS, graduate student | Ph.D. Orals committee | Ph.D. awarded 2005 |
| 2002 | Andre Whitkin | MSTP student, Cornell University USA | Supervised Summer work | MSTP student, Cornell University |
| 2002 | Karin deVisser | The Netherlands Cancer Institute, The Netherlands | Ph.D. Thesis Reading Committee | Postdoctoral fellow, The Netherlands Cancer Institute |
| 2003 | Cathy Collins | UCSF MSTP student | MSTP Advisor | MSTP student, UCSF |
| 2004 | Eric Tamm | University of British Columbia, Canada | Doctoral Dissertation External Examiner | Postdoctoral fellow, Genentech Inc., |
| 2004 | Annie Hsieh | University of Södertörn, Sweden | Masters Thesis Supervisor | unknown |

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|---|---|---|---|---|
| 2005 | Geoff Benton | UCSF TETRAD/PIBS, graduate student | Ph.D. Orals committee | UCSF TETRAD PhD graduate student |
| 2006 | Morgan Truitt | UCSF BMS, graduate student | Rotation Supervisor | UCSF BMS PhD graduate student |
| 2006 | Danielle Shin | UCSF MSTP student | Rotation Supervisor | MSTP student, UCSF |
| 2006-2008 | Celeste Rivera | SFSU/UCSF NIH Post-baccalaureate Research Experience Program (PREP) student | M.S. research advisor | current MS student, Coussens lab UCSF |
| 2007-present | Leslie Vasquez | SFSU/UCSF NIH Post-baccalaureate Research Experience Program (PREP) student | M.S. research advisor | current MS student, Coussens lab UCSF |
| 2008 | Ashley Martin | UCSF BMS, graduate student | Rotation Supervisor | UCSF BMS PhD graduate student |
| 2009 | Kay Wiebrands | Master's Student Utrecht University, the Netherlands | Masters Thesis Internship Supervisor | Master's Student Utrecht University, the Netherlands |
| 2009-2010 | David Tawfik | Medical Student III, UCSF | MSIII break year. Dean's Quarterly Research Fellowship; PACCTR Fall Quarter Fellowship; | MSIII, UCSF |
| 2009-present | Renee Vanderlaan | UCSF BMS, graduate student | Chair: Thesis Committee | UCSF BMS graduate student, Lab of Matthias Hebrook, Ph.D. |
| 2009-present | A. Preethi Ganessan, M.D. | Ph.D. Graduate Student (Cancer Research UK, Univ of Southampton) | Ph.D. supervisor | Current Ph.D. student, Coussens lab |
| 2010-present | Lucia Cottone | Ph.D. graduate student (San Rafaelle Institute, Milan Italy) | Ph.D. supervisor | Current Ph.D. student, Coussens lab |

## Postdoctoral Fellows and Residents Directly Supervised or Mentored

| Dates | Name | Position & Funding | Faculty Role | Current Position |
|---|---|---|---|---|
| 2000 - 2001 | Ernst Lengyel, M.D., Ph.D. | Post-Doc Researcher, Senior Clinical Fellow | Research Supervisor | Assoc. Adj. Prof., Dept. Gyn. & Oncology, UCSF |
| 2000 -2002 | Leon Van Kempen, Ph.D. | Post-Doc Researcher, Dutch Cancer Society Postdoctoral Fellowship | Research Supervisor | Asst Prof., Univ. of Nijmegen, Dept. of Pathology, The Netherlands |
| 2002 – 2005 | Robert Diaz, Ph.D. | Post-Doc Researcher; Coussens R01 | Research Supervisor | Scientist, Roche Pharmaceuticals |
| 2002 – 2005 | Karin deVisser, Ph.D. | Post-Doc Researcher, Dutch Cancer Society Postdoctoral Fellowship | Research Supervisor | Research Scientist, The Netherlands Cancer Institute, Amsterdam, The Netherlands |
| 2003 – 2007 | Alexandra Eichten, Ph.D. | Post-Doc Researcher, Serono Fndt for the Advancement of Medical Science (2003-2005); | Research Supervisor | Scientist, Regeneron Corp., New York USA |
| 2003 - 2005 | Stephen Robinson, Ph.D. | Post-Doc Researcher; Coussens R01 | Research Supervisor | Private sector, United Kingdom |
| 2003 - 2004 | H. Jennifer Shen, Ph.D. | Post-Doc Researcher; Coussens R01 | Research Supervisor | Post-Doctoral fellow, NIH |
| 2005 - | David DeNardo, Ph.D. | Post-Doc Researcher; | Research | Post-Doctoral fellow, |

| | | | | |
|---|---|---|---|---|
| present | | 1) NGA: 5 T32 CA09043 PI: BISHOP; *Molecular Analysis of Tumor Viruses;* 2) American Cancer Society Postdoctoral Fellowship 2007-2010 | Supervisor | Coussens Lab, UCSF |
| 2005 –2007 | Nor Eddine Sounni, Ph.D. | Post-Doc Researcher; Coussens R01 | Research Supervisor | Research Scientist, Univ. of Liege, Belgium |
| 2006 –2007 | Tingting Tan, M.D.,Ph.D. | Post-Doc Researcher; Coussens R01 | Research Supervisor | Resident, Internal Medicine, Kaiser San Francisco |
| 2006 - present | Magnus Johansson, Ph.D. | Post-Doc Researcher; Sweedish Cancer Society Postdoctoral fellowship 2006-2008 | Research Supervisor | Post-Doctoral fellow, Coussens Lab, UCSF |
| 2006- present | Nessrine Affara, Ph.D. | Post-Doc Researcher; AACR-Astellas USA Fndt in Basic Cancer Research 2009-2010 | Research Supervisor | Post-Doctoral fellow, Coussens Lab, UCSF |
| 2007 -2010 | Pauline Andreu, Ph.D. | Post-Doc Researcher; Cancer Research Institute Postdoctoral Fellowship 2008-2011 | Research Supervisor | Research Funding Agency in Private Sector, France |
| 2008- present | Brian Ruffell, Ph.D. | Post-Doc Researcher; Dept of Defense Postdoctoral fellowship 2009-2012 | Research Supervisor | Post-Doctoral fellow, Coussens Lab, UCSF |
| 2009- present | Stephen Shiao, M.D., Ph.D. | UCSF Radiation Oncology Resident | Research Supervisor | UCSF Radiation Oncology Fellowship |
| | | | | |

## FACULTY MENTORING

Faculty Mentored:

| Dates | Name | Position while Mentored | Mentoring Role | Current Position |
|---|---|---|---|---|
| 2001 – 2004 | Ernst Lengyel, M.D., Ph.D. | Asst. Adjunct Professor | Research Mentor | Asst. Prof., Dept. Gyn. & Oncology, Univ. of Chicago, Chicago, IL |
| 2002 – 2007 | Darya Soto, M.D. | Asst. Adjunct Professor, | K08 Research Mentor | Private Practice, Burlingame, CA |
| 2005 – 2007 | Runi Chattopadhyay, M.D. | Clinical Instructor and Clinical Fellow | Basic Science Mentor, K12 | Director, Breast Center, California Pacific Med. Center, San Francisco CA |
| 2006 – 2009 | Limin Liu, Ph.D. | Assistant Professor | Member, Mentoring Committee | Dept. of Microbiology & Immunology, Sandler Center for Basic Research in Asthma, UCSF |
| 2010-present | Jaynata Debnath, M.D., Ph.D., | Assistant Professor | Faculty Mentor | Dept. of Pathology, UCSF |

Sabbatical Visitors:

1999 - 2000 Yves DeClerck, M.D. Professor, Univ. of Southern Calif. & Children's Hospital of Los Angeles

## SUMMARY OF TEACHING HOURS

| Academic Year | Teaching/Mentoring Summary | Hours |
|---|---|---|
| 1997/98 | Total hours of teaching /mentoring:<br>Formal class or course teaching hours:<br>Informal teaching hours including prep time:<br>Mentoring hours: | 27<br>2<br>1<br>24 |
| 1998/99 | Total hours of teaching /mentoring:<br>Formal class or course teaching hours:<br>Informal teaching hours including prep time:<br>Mentoring hours: | 71<br>2<br>1<br>68 |
| 1999/00 | Total hours of teaching /mentoring:<br>Formal class or course teaching hours:<br>Informal teaching hours including prep time:<br>Mentoring hours: | 108<br>4<br>2<br>102 |
| 2000/01 | Total hours of teaching /mentoring:<br>Formal class or course teaching hours:<br>Informal teaching hours including prep time:<br>Mentoring hours: | 130<br>16<br>9<br>105 |
| 2001/02 | Total hours of teaching /mentoring:<br>Formal class or course teaching hours:<br>Informal teaching hours including prep time:<br>Mentoring hours: | 201<br>18<br>19<br>164 |
| 2002/03 | Total hours of teaching /mentoring:<br>Formal class or course teaching hours:<br>Informal teaching hours including prep time:<br>Mentoring hours: | 314.5<br>15.5<br>17<br>282 |
| 2003/04 | Total hours of teaching /mentoring:<br>Formal class or course teaching hours:<br>Informal teaching hours including prep time:<br>Mentoring hours: | 402<br>20<br>28<br>354 |
| 2004/05 | Total hours of teaching /mentoring:<br>Formal class or course teaching hours:<br>Informal teaching hours including prep time:<br>Mentoring hours: | 395<br>17<br>28<br>350 |
| 2005/06 | Total hours of teaching /mentoring:<br>Formal class or course teaching hours:<br>Informal teaching hours including prep | 395<br>17<br>28 |

| | | |
|---|---|---|
| | time:<br>Mentoring hours: | 350 |
| 2006/2007 | Total hours of teaching /mentoring:<br>Formal class or course teaching hours:<br>Informal teaching hours including prep time:<br>Mentoring hours | 473<br>45<br>28<br>400 |
| 2008/2009 | Total hours of teaching /mentoring:<br>Formal class or course teaching hours:<br>Informal teaching hours including prep time:<br>Mentoring hours | 499<br>51<br>48<br>400 |
| 2009/2010 | Total hours of teaching /mentoring:<br>Formal class or course teaching hours:<br>Informal teaching hours including prep time:<br>Mentoring hours | 499<br>51<br>48<br>400 |
| 2010/2011 | Total hours of teaching /mentoring:<br>Formal class or course teaching hours:<br>Informal teaching hours including prep time:<br>Mentoring hours | 499<br>51<br>48<br>400 |

## X. RESEARCH AND CREATIVE ACTIVITIES

### RESEARCH AWARDS AND GRANTS:

**CURRENT**

**R01CA140943** (multi-PI: Coussens, Boudreau, Daldrup-Link; UCSF) 07/01/09 –06/30/13
**Source**: NIH/NCI
**Title**: Improved Imaging and Drug Delivery Using Novel Approaches to Regulate Tissue Perfusion
The major goal of this project is to examine how short-term inhibition of ALK5 in vivo alters hemodynamics and tissue perfusion in mouse models of cancer.
**Role**: P.I.

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**RO1 CA130980** (PI: Coussens, LM; UCSF) 07/01/08-06/30/13
**Source**: NIH/NCI
**Title**: Regulation of Inflammation-Associated Epithelial Cancer Development
**Role**: Principal Investigator
The goal of this study is to determine regulatory programs activating chronic inflammation during squamous carcinogenesis

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**RO1 CA132566** (multi PI: Coussens, LM; Jablons DM; UCSF) 05/01/08-04/30/13
**Source**: NIH/NCI
**Title**: Inflammation and Lung Carcinogenesis
**Role**: Principal Investigator
The goal of this study is to determine how inflammation and Wnt signaling regulate stem cell

niche autonomy during lung carcinogenesis

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**W81XWH-08-PRMRP-IIRA** (multi-PI: Broaddus, C; Coussens, LM; UCSF) 07/01/09 –06/30/12
**Source**: DOD
**Title**: Role of Macrophage-induced Inflammation in Mesothelioma
The goals of this project are 1) to determine the functional significance of macrophage phenotype in mesothelioma, 2) to determine the functional significance of macrophages as regulators of mesothelioma apoptosis in vitro and 3) to define the functional significance of macrophage depletion or repolarization on mesothelioma survival in vivo.
**Role**: P.I.

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**P50 CA58207** (Gray; LBNL/UCSF) 08/01/92–11/30/12
**Source**: NIH/NCI
**Title**: Bay Area Breast Cancer SPORE
Career Development and Developmental Research Award, Multi Project PI: Boudreau N; Coussens LM
Title: Macrophage-Mediated Delivery of the Breast Tumor Suppressor HoxD10 via Autologous Transfer to Breast Tumors. The aims of this project are to 1) establish function and optimize introduction of the engineered HoxD10 protein into macrophages and/or monocytes; 2) visualization of modified monocyte/macrophage accumulation in mammary tumors in vivo and 3) analysis of the impact of monocyte/macrophage delivered HoxD10 on breast tumor growth, progression and metastasis in MMTV-PyMT mouse model of mammary carcinogenesis.
**Role**: Multi P.I.

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**P30 CA82103** (PI: McCormick, F; UCSF) 08/5/99-05/31/12
**Source:** NIH/NCI
**Title:** *Cancer Center Support Grant*
**Role**: Co-Director, Mouse Pathology Shared Resource
The Cancer Center Support Grant provides support for administration and infrastructure for the UCSF Comprehensive Cancer Center. Dr. Coussens is the Core Co-Director of the Mouse Pathology Shared Resource that provides routine hematologic and histopathologic processing of tissue and blood samples to members of the UCSF community.

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**BC051640 Era of Hope Scholar Award** (PI: Coussens, LM; UCSF) 06/01/06 – 05/31/11
**Source:** Department of Defense Breast Cancer Research Program (BCRP) of the Office of the Congressionally Directed Medical Research Programs (CDMRP**)**

**Title**: Microenvironment Regulation of Mammary Carcinogenesis
**Role**: PI
The goal of this Scholar Award is to identify leukocytes and their proteases that modify breast carcinogenesis and to develop noninvasive imaging reagents targeting leukocytes to image inflammation.

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**PREVIOUS**
**USPHS 5 T32 CA09056** (PI: Fox, F, UCLA) 07/01/89 –06/30/92
**Source:** NIH/UCLA
Title: Regulation of *junB* Gene Expression by TGF-Beta

Competitive Pre-Doctoral award to study transcription factor *junB.*

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**Univ. of Calif., Dissertation Year Fellowship** (PI: Coussens, LM, UCLA) 10/1/92 – 09/31/93
**Source:** University of California, Office of the President
**Title**: *Effects of E1A on TGF-Beta-inducible junB Expression*
Competitive Pre-Doctoral award to study transcription factor *junB.*

**USPHS 5 T32 CA09043** (PI: Bishop, KM, UCSF) 10/01/93-06/31/96
**Source:** NIH/UCSF
**Title**: *Molecular Analysis of Tumor Viruses*
Post-Doctoral fellowship to study mouse model of epithelial carcinogenesis.

**American Social Health Association/Pfizer Post-Doctoral Research Fellowship in Sexually Transmitted Diseases** (PI: Coussens, LM, UCSF) 10/01/96 – 9/30/98
**Source:** Private Foundation
**Title**: *Metalloproteinases and Malignant Progression of Squamous Epithelium in K14-HPV16 Transgenic Mice*
**Role**: Principal Investigator
Competitive Post-Doctoral fellowship to study proteases and tumor development .

**P01 CA072006** (PI: Shuman M, UCSF) 06/10/97 – 06/30/03
**Source:** NIH/NCI
**Title**: *Proteases in Cancer Biology and Drug Development*
Project 3 – Proteases in Models of Tumor Initiation/Progression
**Role**: Co-Investigator, Project 3
The major goal of this project is to study the role of proteases in cancer biology.

Core C – Transgenic Animal Models
**Role**: Director (year 4 and 5)
The major goal of this Core is to develop and provide protease null and transgenic mice to program projects.

**UCSF IRG-97-150-01** (PI: Coussens LM, UCSF) 07/01/99-06/30/00
**Source:** American Cancer Society
**Title***: Proteases and Genomics in a Mouse Model of Epithelial Cancer*
**Role**: Principal Investigator
Pilot project tested role of proteinases as effectors of genomic instability.

**UCSF Cell Cycle and Dysregulation Program** (PI: Coussens LM, UCSF) 02/01/00-01/31/01
**Source:** UCSF Comprehensive Cancer Center, Intramural
**Title***: Epithelial Neoplastic Progression and Degradation of Type I Collagen*

**Role**: Principal Investigator
Pilot project assessed functional significance of type I collagen metabolism during epithelial carcinogenesis.

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**Research Evaluation and Allocation Committee** (PI: Coussens LM, UCSF) 07/01/00-06/30/01
**Source:** UCSF Academic Senate
**Title**: *Role of Gelatinase B in Maintenance of Genomic Instability*
**Role**: Principal Investigator
Pilot project tested the role of MMP9 as an indirect regulator of genomic instability.

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**UCSF IRG AC-04-02** (PI: Coussens LM, UCSF) 10/1/00-09/30/01
**Source:** American Cancer Society
**Title**: *Regulation of Intracellular Signaling Pathways by Gelatinase B/MMP-9*
**Role**: Principal Investigator
Pilot project to study signal transduction pathways regulated by MMP-9.

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**The V Foundation for Cancer Research** (PI: Coussens LM, UCSF) 06/02/00-05/31/02
**Source:** Private Foundation
**Title***: Gelatinase B and Epithelial Cancer Development*
**Role**: Principal Investigator
Pilot project to study role of MMP9 during epithelial carcinogenesis.

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**Gertrude B. Elion Cancer Research Award** (PI: Coussens LM, UCSF) 07/1/01 – 06/30/02
**Source:** American Association of Cancer Research
**Title**: *Functional Role of MMP-2 During Epithelial Carcinogenesis*
**Role**: Principal Investigator
Pilot project to study role of MMP-2 during epithelial carcinogenesis.

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**Univ. of Calif., Cancer Research Coordinating Committee** (PI: Coussens LM, UCSF) 07/01/01 – 06/30/02
**Source:** University of California
**Title**: *Gelatinase A/MMP-2 and Epithelial Cancer Development*
**Role**: Principal Investigator
Pilot project to study role of MMP-2 as a potentiator of tumor development.

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**Hellman Family Award For Early Career Faculty** (PI: Coussens LM, UCSF) 11/01/00-09/30/02
**Source:** UCSF Intramural
**Title**: *Paracrine Regulation of Epithelial Carcinogenesis by MMP-9*
**Role**: Principal Investigator
Pilot project to identify matrix molecules regulated by MMP-9.

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**Edward Mallinckrodt, Jr. Foundation** (PI: Coussens LM, UCSF) 10/01/00-09/30/03
**Source:** Private Foundation
**Title**: *Regulation of epithelial cancer by gelatinase B/MMP-9*

**Role**: Principal Investigator
Pilot project to determine how MMP-9 regulates proliferation, VEGF bioavailability and angiogenesis during epithelial carcinogenesis.

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**P50 CA58207** (PI: Gray, J: UCSF) 03/01/03-02/28/05
**Source:** NIH/NCI
Bay Area Breast Cancer Translational Research Program (SPORE)
**Title**: *Type I Collagen Remodeling and Mammary Carcinogenesis*
**Role**: Principal Investigator (Developmental Project)
The overall goal of this pilot project was to explore the role of collagen metabolism during mammary carcinogenesis.

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**DE-FG02-05ER6401 (**PI: Franc, B**;** UCSF**)** 03/01/05 – 01/16/06
**Source:** DOE Medical Applications Grant
**Title**: Therapeutic Radionuclide Tumor-targeting Strategy for Breast Cancer

directs

**Role**: Co-Investigator
The specific aim of this project is to develop a radionuclide delivery molecule (RDM) that specifically targets cancer cells that express matrix-metalloproteinase-14 (MMP-14) on their surface and demonstrate delivery of radiolabeled RDM to MMP-14 expressing cells *in vitro* and *in vivo.*

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**R01 DK067678** (PI: Cher, M: Wayne State University) 07/01/03-06/30/06
**Source:** NIH/NIDDK
**Title**: *Proteases in Prostate Cancer Bone Metastasis*
**Role**: Subcontract Principal Investigator
The major goal of this subcontract is to assist with the planned experiments by providing mice (protease deficient) of defined genotype for proposed studies to analyze proteases during prostate metastasis to bone *in vivo*.

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**Opportunity Award, Sandler Family** (PI: Coussens, LM; UCSF) 02/15/05 -02/14/07
**Source:** UCSF Intramural
**Title**: *B Lymphocytes as Targets for Cancer Prevention*
**Role**: Principal Investigator
The major goal of this project is to investigate the efficacy of targeting B cells for chemoprevention

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**DAMD17-02-1-0693** (PI: Sloane, B; Wayne State University) 08/01/02-07/31/06
**Source:** Department of Defense
***Breast Cancer Center of Excellence***
**Title**: *Validation of Proteases as Therapeutic Targets in Breast Cancer Functional Imaging of Protease Expression, Activity and Inhibition*
**Role**: Subcontract Principal Investigator
The goal of this program is to validate proteases as therapeutic targets in breast cancer by functional imaging of protease expression, activity and inhibition.

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**R01 CA94168** (PI: Coussens, LM: UCSF) 04/01/02-06/31/07
**Source:** NIH/NCI
**Title**: *Regulation of Epithelial Cancer by MMP-9/gelatinase B*

**Role**: Principal Investigator
The goal of this project is to identify molecules that mediate proliferative and cellular pathways activated by MMP-9.

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**U54 RR020843** (PI: Smith, J; Burnham Institute) 09/30/04-07/31/06
**Source:** NIH/National Center for Research Resources

**Title**: Center on Proteolytic Pathways
**Role**: Principal Investigator (Driving Biological Problem #1)
DBP#1 *Proteolytic Pathways in Acute Vascular Response*

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**P01 CA72006** (PI: Werb, Z; UCSF) 07/07/03 – 06/30/08
**Source:** NIH/NCI
**Title**: *Proteases in Cancer Biology and Drug Development* -
Project 3 - *Proteases in Models of Tumor Initiation/Progression*
**Role**: Co-Investigator, Project 3 -
The major goal of this project is to study the role of proteases in cancer biology.
Core C - *Transgenic Animal Models*
**Role**: Director
The major goal of this Core is to develop and provide protease null and transgenic mice to program projects.

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**R01 CA98075** (PI: Coussens, LM; UCSF) 07/01/03-06/30/09
**Source:** NIH/NCI
**Title**: *Microenvironmental Regulation of Tumor Progression*
**Role**: Principal Investigator

The overall goal of this grant is to determine the role of collagen metabolism on epithelial carcinogenesis.

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## XI. PEER REVIEWED PUBLICATIONS

1. Francke U, de Martinville B, **Coussens L**, Ullrich A. (1983) The human gene for the Beta subunit of nerve growth factor is located on the proximal short arm of chromosome 1. *Science* 222:1248-1251.
2. Breakefield X, Castiglione C, **Coussens L**, Axelrod F, Ullrich A. (1984) Structural gene for Beta-nerve growth factor is not defective in familial dysautonomia. *Proc. Natl. Acad. Sci. USA* 81:4213-4216.
3. Ullrich A, **Coussens L**, Hayflick J, Dull T, Gray A, Tam A, Lee J, Yarden Y, Libermann T, Schlessinger J, Downward J, Bye J, Whittle N, Waterfield M, Seeburg P. (1984) Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. *Nature* 309:418-425.
4. Ullrich A, Bell J, Chen E, Herrera R, Petruzzelli L, Dull T, Gray A, **Coussens L**, Liao Y-C, Tsubokawa M, Mason A, Seeburg P, Grunfield C, Rosen O, Ramachandran J. (1985) Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. *Nature* 313:756-761.
5. Lauffer L, Garcia P, Harkins R, **Coussens L**, Ullrich A, Walter P. (1985) Topology of the signal

recognition particle receptor in the endoplasmic reticulum membrane. *Nature* 318:334-338.

6. Schechter A, Hung M-C, Vaidanathan L, Weinberg R, Yang-Feng T, Francke U, Ullrich A, **Coussens L.** (1985) The *neu* gene: An *erbB*-homologous gene distinct from and unlinked to the gene encoding the EGF receptor. *Science* 229:976-978.
7. **Coussens L**, Yang-Feng T, Liao T-C, Chen E, Gray A, McGrath J, Seeburg P, Libermann T, Schlessinger J, Francke U, Levinson A, Ullrich A. (1985) Tyrosine kinase receptor with extensive homology to the EGF receptor shares chromosomal location with *neu* oncogene. *Science* 230:1132-1139.
8. **Coussens L**, Van Beveren C, Smith D, Chen E, Mitchell R, Isacke C, Verma I, Ullrich A. (1986) Structural alteration of viral homologue of receptor proto-oncogene *fms* at carboxyl terminus. *Nature* 320:277-280.
9. Parker P, **Coussens L**, Totty N, Rhee L, Young S, Chen E, Stabel S, Waterfield M, Ullrich A. (1986) The complete primary structure of protein kinase C—the major phorbol ester receptor. *Science* 233:853-859.
10. **Coussens L**, Parker P, Rhee L, Yang-Feng T, Chen E, Waterfield M, Francke U, Ullrich A. (1986) Multiple, distinct forms of bovine and human protein kinase C suggest diversity in cellular signaling pathways. *Science* 233:859-866.
11. **Coussens L**, Rhee L, Parker P, Ullrich A. (1987) Alternative splicing increases the diversity of the human protein kinase C family. *DNA* 6:389-394.
12. Yarden Y, Kuang W-J, Yang-Feng T, **Coussens L**, Munemitsu S, Dull T, Schlessinger J, Francke U, Ullrich A. (1987) Human proto-oncogene *c-kit*: A new cell surface receptor-tyrosine kinase for an unidentified ligand. *EMBO J*. 6:3341-3351.
13. MacDonald R, Pfeffer S, **Coussens L**, Tepper M, Brocklebank C, Mole J, Anderson J, Chen E, Czech M, Ullrich A. (1988) A single receptor binds both insulin-like growth factor II and mannose-6-phosphate. *Science* 239:1134-1137.
14. Formby B, Ullrich A, **Coussens L**, Walker L, Peterson C. (1988) Growth hormone stimulates insulin gene expression in cultured human fetal pancreatic islets. *J. Clin. Endo. Metab.* 66:1075-1079.
15. Mosthaf L, Grako D, Dull T, **Coussens L**, Ullrich A, McClain D. (1990) Functionally distinct insulin receptors generated by tissue-specific alternative splicing. *EMBO J.* 9:2409-2413.
16. **Coussens LM**, Yokoyama K, Chiu R. (1994) Transforming Growth Factor $b_1$-mediated induction of *junB* is selectively inhibited by expression of Ad.12-E1A. *J. Cell. Physio.* 160:435-444.
17. **Coussens LM**, Hanahan D, Arbeit J. (1996) Genetic predisposition and parameters of malignant progression in K14-HPV16 transgenic mice. *Am. J. Pathol.* 149:1899-1917.
18. **Coussens LM**, Werb Z. (1996) Matrix metalloproteinases and the development of cancer. *Chemistry and Biology* 3:895-904.
19. Bergers G, Hanahan D, **Coussens LM.** (1998) Angiogenesis and apoptosis are cellular parameters regulating neoplastic progression in transgenic mouse models of tumorigenesis. *Int. J. Dev. Biol. 42*:995-1002.
20. **Coussens LM,** Raymond WW, Bergers G, Laig-Webster M, Behrendtsen O, Werb Z, Caughey, GH, Hanahan D. (1999) Inflammatory mast cells upregulate angiogenesis during squamous epithelial carcinogenesis. *Genes & Development* 13:1382-1397.
21. McKerrow JM, Bhargava V, Hansell E, Kuwahara T, Matley M, **Coussens LM,** Warren R. (2000) A functional proteomics screen of proteases in colorectal carcinomas. *Molecular Medicine* 6:450-460.
22. Bergers G, **Coussens LM.** (2000) Extrinsic regulators of epithelial tumor progression: metalloproteinases. *Curr. Opin. Genetics & Development* 10:120-127.
23. **Coussens LM,** Tinkle CL, Hanahan DH, Werb Z. (2000) MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. *Cell* 103:481-490.

24. van Kempen LCL, Rhee JS, Dehne K, Lee J, Edwards DR, **Coussens LM**. (2002) Epithelial carcinogenesis: Dynamic interplay between neoplastic cells and their microenvironment. *Differentiation* 70: 610-623.
25. **Coussens LM**, Fingleton B, Matrisian LM. (2002) Matrix metalloproteinases and cancer: Trials and tribulations. *Science* 295:2387-2392.
26. **Coussens LM**, Werb Z. (2002) Inflammation and cancer. *Nature* 420: 860-867.
27. Daniel D, Meyer-Morse N, Bergsland EK, Dehne K, **Coussens LM,** Hanahan D. (2003) Immune enhancement of skin carcinogenesis by $CD4^+$ T cells. *J Exp. Med*, 197:1017-1028.
28. van Kempan LCL, Ruitter DJ, van Muijen GNP, **Coussens LM.** (2003) The tumor microenvironment: a critical determinant of neoplastic evolution. *Euro J Cell Biol,* 82:539-548.
29. Rhee JS, Diaz R, Korets L, Hogson G, **Coussens LM**. (2004) TIMP-1 alters susceptibility to carcinogenesis. *Cancer Research* 64:952-961.
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31. Baluk P, Raymond WW, Ator E, **Coussens LM**, McDonald DM, Caughey GH. (2004) Matrix metalloproteinase-2 and -9 expression increases in mycoplasma-infected airways but is not required for vascular remodeling. *Am J Physiol Lung Cell Mol Physiol*, 287:307-317.
32. de Visser KE, Korets LV, **Coussens LM**. (2004) Early neoplastic progression is complement-independent. *Neoplasia* 6: 768-776.
33. Jodele S, Chantrain CF, Blavier L, Crooks GM, Shimada H, **Coussens LM**, DeClerck YA. (2005) The contribution of bone marrow-derived cells to the tumor vasculature in neuroblastoma is matrix metalloproteinase-9-dependent. *Cancer Research,* 65: 3200-3208.
34. de Visser KE, Korets LV, **Coussens LM**. (2005) De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. *Cancer Cell* 7:411-423.

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- *Faculty of 1000 – Exceptional (Factor 6.0).* Authoritative evaluation by R. Abraham, Wyeth Research. http://f1000biology.com/guardpages/evaluation/1015124//
- *Nature (2005*), 435:752-753. 'Inflammation by Remote Control'. A. Mantovani
- *Cancer Cell (*2005), 7: 403-405. 'The role of the immune system in early carcinogenesis: B-ware the double edged sword'. A. Houghton, H. Uchi, and J.D. Wolchok.
- *Nature Reviews Immunology (2005)*, 5:517. 'B cells lead the way in tumour progression'. K. Minton
- *Nature Reviews Cancer (2005)*, 5:501. 'B cells lead the way in tumour progression'. K. Minton

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36. de Visser KE, Eichten A, **Coussens LM**. (2006) Paradoxical roles of the immune system during cancer development. *Nature Reviews Cancer*, 6:24-37.
37. van Kempen LCL, de Visser KE, **Coussens LM**. (2006) Inflammation, Proteases and Cancer. *Euro J Cancer,* 42: 728-734.
38. Yuan F, Verhelst HL, Blum G, **Coussens LM**, Bogyo M. (2006) A selective activity-based probe for the papain family cysteine protease dipeptidyl peptidase I/Cathepsin C. *J Am Chem Society*, 128: 5616-5617.
39. Tan TT, **Coussens LM**. Humoral immunity, inflammation and cancer. (2007) *Curr Opin Immunology* 19(2), 209-216
40. Johansson M, Tan T, de Visser KE, **Coussens LM**. (2007) Immune cells as anti-cancer therapeutic targets and tools. *J Cellular Biochemistry*, 101: 918-926.
41. Schwartz DR, Moin K, Yao B, Matrisian LM, **Coussens LM**, Bugge TH, Fingleton B, Acuff KB,

Sinnamon M, Nassar H, Krawetz SA, Linebaug BE, Sloane, BF. (2007) Hu/Mu ProtIn oligonucleotide microarray: dual species array for profiling protease and protease inhibitor gene expression in tumors and their microenvironment. *Mol Cancer Res,* 5:443-454.

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43. Eichten AE, Hyun WC, **Coussens LM**. (2007) Distinctive features of angiogenesis and lymphangiogenesis determine their functionality during de novo tumor development. *Cancer Research,* 67:5211-5220. PMID: 17545601
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46. Kenny H, Kaur S, **Coussens LM**, Lengyel E. (2008) Adhesion of OvCa cells to peritoneum is mediated by MMP-2 cleavage of fibronectin*, J Clin Invest.* 118(4)1367-1379. PMCID: PMC2267016
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- *Faculty of 1000 – Exceptional (Factor 9.0).* Authoritative evaluation by A. Mantovani, Univ. of Milan. http://f1000biology.com/guardpages/evaluation/1163484//
- *Cancer Cell* (2009), 16:81-82. 'Metastasis-Promoting Immunity: When T cells turn to the dark side' by D. Pardoll
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- *Nature Biotechnology* (2009) 27:828. 'Promoting metastasis' by L. DeFrancesco
- *Nature Medicine* (2009) 15:1135. 'Not Immune' by C. Schubert

**49.** Sista AK, Knebel RJ, Tavri SA, Johansson M, DeNardo DG, Boddington SE, Kishore SA, Ansari C, Reinhart V, Coakley FV, **Coussens LM**, Daldrup-Link HE. (2009) Optical Imaging of the peri-tumoral inflammatory response in breast cancer. *J Transl Med*, 7(1): 94-100. PMCID: PMC2780997

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- *Cancer Cell* (2010), 17(2) 111-112. '*La mala educacion of tumor-associated macrophages: Diverse pathways and new players',* by A. Mantovani
- *Nature Reviews Immunology* (2010) 10:158 'Antibodies lend support to tumors', by, L. Byrd
- *Faculty of 1000 – Exceptional (Factor 6.4).* Authoritative evaluations by X. Ma (Weill

Medical College of Cornell) and F. Kiefer (Max-Planck-Institute, Germany). http://f1000biology.com/article/id/2467956

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53. Masset A, Maillard C, Sounni NE, Jacobs N, Bruyere F, Delvenne P, Tacke M, Reinheckel T, Foidart JM, **Coussens LM**, Noel A. Unimpeded skin carcinogenesis in K14-HPV16 transgenic mice deficient for plasminogen activator inhibitor. *Int J Cancer*, Mar 15. *[Epub ahead of print]* PMID: 20232379

***Manuscripts Submitted or In Revision:***

- Egeblad M, Wiseman BS, Sternlicht MD, Green KA, Kouros-Mehr H, DeNardo D, Wilcox J, Bissel MJ, **Coussens LM**, Lund LR, Werb Z. Matrix metalloproteinase-dependent remodeling of the collagen scaffold regulates mammary epithelial invasion. *Manuscript submitted*
- Okamoto J, Mikami I, Raz DJ, Segal M, Yagui-Beltran A, Johansson A, **Coussens LM**, Chen Z, Zhou HM, Hirata T, Clement G, Koizumi K, Shimizu K, Jablons DM, He B. Down regulation of EMX2 is associated with clinical outcome in lung adenocarcinoma, *Manuscript submitted*

## XII. PATENTS

1. U.S. Patent Application Serial No. 10/567,873
   Title: *Novel Indications for Transforming Growth Factor-Beta Regulators*.
   Inventors: **Lisa M. Coussens** and Zena Werb
   Application published on August 28, 2008 as U.S. Patent Publication #2008-0206219-A1
   International filing date: August 9, 2004. Application Filing Number: 60/493,643; Docket Number: 23540-09361/PCT; International Publication Number WO 2005/013915 A2; International Application Number: PCT/US2004/025902
2. DeNardo D, Brennan D, **Coussens LM**. *Phenotyping Tumor-Infiltrating Leukocytes*, Provisional patent application number: 61/227,035

## XIII. NON-PEER REVIEWED PUBLICATIONS AND EDITORIALS:

**Review Articles, Symposium Proceedings and Editorials**

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2. Ullrich A, Riedel H, Yarden Y, **Coussens LM**, Gray A, Dull T, Schlessinger J, Waterfield MD, Parker PJ. (1986) Protein kinases in cellular signal transduction: Tyrosine kinase growth factor receptors and protein kinase C. *CSHSQ* 51:713-724.
3. Chen E, **Coussens LM**, Liao Y-C, Smith D, Yang-Feng T, McGrath J, Van Beveren C, Verma IM, Libermann TA, Schlessinger J, Francke U, Levinson A, Ullrich A. (1986) Structural features ofgrowth factor receptors with oncogenic potential. *Biochem. Soc. Symp*. 52:65-82.
4. **Coussens LM,** Werb Z. (2001) Inflammatory cells and cancer: Think Different! *J. Exp. Med.* 193:F23-F26.
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development. *Trends in Cell Biology*, *12:* 209-211.

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9. Robinson S, **Coussens LM**. (2005) Soluble mediators of inflammation during tumor development. *Adv Cancer Research* 93:159-187.
10. Fingleton B, **Coussens LM**. (2005) Host-tumor interactions influencing cancer progression. *Drug Discovery Today: Disease Mechanisms*, 2:199-204
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## XIV. RESEARCH PROGRAM:

The Coussens lab focuses on the role of immune cells and leukocyte proteases as critical

regulators of skin, lung and breast cancer development. During the early development of cancer, many physiological processes occur in the vicinity of 'young tumor cells' that are similar to processes that occur during embryonic development and to healing of wounds in adult tissue, e.g., inflammation, angiogenesis (development of new blood supply) and tissue remodeling. During tumor development; however, instead of initiating a 'healing' response, activated leukocytes provide growth-promoting factors that help tumors grow. We are interested in understanding the molecular mechanisms that regulate leukocyte recruitment into neoplastic tissue and subsequent regulation those leukocytes exert on evolving cancer cells. To address these issues, we have taken several approaches to investigate mechanisms involved in: *i.* induction and maintenance of chronic inflammatory microenvironments in premalignant, malignant and metastatic tissues, *ii.* role of leukocyte proteases as regulators of tissue remodeling, angiogenesis and cancer development, and *iii.* development of novel non-invasive imaging reagents to monitor immune response in tissues/tumors. The long-term goal of this work is to translate basic observations made in the mouse, toward rational design of novel therapeutics whose aim will be to block and/or alter rate-limiting events critical for solid tumor growth or maintenance in humans. Currently, we are actively utilizing transgenic mouse models of cancer development, including non-small cell lung cancer, skin and breast cancer, and mesothelioma to reveal the functional roles of adaptive and innate leukocytes during solid tumor development. These experimental studies are conducted in parallel with evaluation of representative human cancer specimens to affirm that mechanisms revealed in the experimental setting represent fundamental parameters of multi-stage cancer development in humans.

## XV. MOST SIGNIFICNT PUBLICATIONS

1. de Visser KE, Korets LV, **Coussens LM**. (2004) Early neoplastic progression is complement-independent. *Neoplasia* 6: 768-776.

**Role:** This study was based on the hypothesis that inflammation accompanying premalignant progression in HPV16 mice was dependent upon activation and mobilization of the Complement cascade. To address this, my laboratory generated Complement protein 3 (C3)-deficient HPV16 mice and assessed the functional significance of C3 by its absence during neoplastic progression. These studies revealed that abundant deposition of C3 is a characteristic feature of premalignant hyperplasias and dysplasias coincident with leukocyte infiltration in neoplastic tissue, genetic elimination of C3 neither affects inflammatory cell recruitment towards neoplastic skin, nor impacts responding pathways downstream of inflammatory cell activation, e.g. keratinocyte hyperproliferation or angiogenesis. Taken together, these data suggest that complement-independent pathways are critical for leukocyte recruitment into neoplastic tissue and leukocyte-mediated potentiation of tumorigenesis. 100% of the research supporting this manuscript was conceived and conducted in my laboratory. de Visser wrote the manuscript under Dr. Coussens' direct supervision.

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2. de Visser KE, Korets LV, **Coussens LM**. (2005) De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. *Cancer Cell,* 7:411-423.

**Role:** Chronic inflammation predisposes tissue to cancer development; however, regulatory mechanisms underlying recruitment of innate leukocytes toward developing neoplasms are obscure. In this manuscript, we reported that genetic elimination of mature T and B lymphocytes in HPV16 mice, limits neoplastic progression to development of epithelial hyperplasias that fail to recruit innate immune cells. Adoptive transfer of B lymphocytes or serum from HPV16 mice into T and B cell-deficient/HPV16 mice restored innate immune cell infiltration into premalignant tissue and reinstated necessary parameters for full malignancy, e.g., chronic inflammation, angiogenic vasculature, hyperproliferative epidermis. These findings support a model in which acquired immunity is required for establishing chronic inflammatory states that promote de novo carcinogenesis. This report received a great deal of attention as it represented the first such report linking adaptive immune responses during the earliest stages of epithelial carcinogenesis - a paradigm shift from current thinking that revealed provocative new targets for anti-cancer therapy. This manuscript was the "featured article" in its issue of *Cancer Cell*, and was the subject of several invited review articles (Houghton et al., *Cancer Cell* 2005; Montavani, *Nature* 2005), as well as being featured in 'Research Highlights' in *Nature Reviews Cancer* and *Nature Reviews Immunology* (Minton, 2005). 100% of the research supporting this manuscript was conceived and conducted in my laboratory. de Visser wrote drafts of the manuscript under Dr. Coussens' direct supervision.

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3. DeNardo DG, Baretto JB, Andreu P, Vasquez L, Kolhatkar N, **Coussens LM.** (2009) $CD4^+$ T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. *Cancer Cell,* 16:91-102.

**Role:** Infiltration of T lymphocytes in human breast cancers has been recognized by pathologist for decades, however their functional role has been undetermined. We utilized a transgenic mouse model of mammary carcinogenesis and demonstrated a tumor-promoting role for $T_H2$-$CD4^+$ T lymphocytes that elicit pro-tumor, as opposed to cytotoxic bioactivities of tumor-associated macrophages and enhancement of pro-metastatic epidermal growth factor receptor signaling programs in malignant mammary epithelial cells. This work revealed a novel pro-tumor regulatory program involving components of the acquired and cellular immune systems that

effectively collaborate to promote pulmonary metastasis of mammary adenocarcinomas, and identified new cellular targets, namely $CD4^+$ T effector cells and IL-4 for anti-cancer therapy. This manuscript appeared as the "featured article" in the August 4th 2009 issue of *Cancer Cell*, and was the subject of an invited "Preview" article (Pardoll, *Cancer Cell* 2009), and was featured in the research highlights section of *Nature Reviews Cancer* (McCarthy, 2009*).* 100% of the research supporting this manuscript was conceived and conducted in my laboratory. Dr. DeNardo wrote drafts of the manuscript under Dr. Coussens' direct supervision.

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4. Andreu P, Johansson M, Affara NI, Tan TT, Junankar S, Korets L, Lam J, Tawfik D, Pucci F, De Palma M, DeNardo D, de Visser KE, **Coussens LM**. (2010) FcRγ activation regulates inflammation-associated squamous carcinogenesis. *Cancer Cell,* 17(2):121-134.

**Role:** This work presents novel findings demonstrating the functional significance of Fcg receptors and humoral immunity as potentiators of squamous carcinogenesis. While myeloid cells and some T cell subsets have been implicated in neoplastic progression and cancer development, the tumor-promoting capabilities of B lymphocytes have remained unclear. Using the HPV16 transgenic mouse model of inflammation-associated squamous carcinogenesis, we previously reported that B and T cell-deficient HPV16 mice failed to progress beyond a benign hyperplastic state due to deficient activation of chronic inflammatory programs in early neoplastic skin (deVisser at al, *Cancer Cell* 2005). In Andreu et al., we revealed revealed that B cells potentiate squamous carcinogenesis via humoral immunity, where immunoglobulins (Ig) in the form of immune complexes (IC) activate Fcγ receptor-mediated signaling pathways on resident and recruited myeloid cells. Activation of these programs on resident mast cells initially leads to peripheral blood leukocyte recruitment into neoplastic skin and activation of angiogenic vasculature. The subsequent chronic inflammation that ensues in part maintains angiogenic support but also supports neoplastic keratinocyte hyperproliferation and progression to dysplastic/carcinoma *in situ* states and subsequent malignant conversion and carcinoma development. These novel findings have clinical significance in that they imply that anti-cancer strategies targeting B cells, Ig or FcRγ may harbor therapeutic efficacy in limiting risk of malignant conversion in patients suffering from chronic inflammatory diseases, or in patients harboring premalignant lesions whose molecular and/or immunologic characteristics favor tumor development. This manuscript appeared as the "featured article" in the March 2010 issue of *Cancer Cell*, and was the subject of an invited "Preview" article (Mantovani, *Cancer Cell* 2010), and was featured in the research highlights section of *Nature Reviews Immunology* (Byrd, 2010*).* 100% of the research supporting this manuscript was conceived and conducted in my laboratory. Dra. Andreu, Johansson and Affara wrote drafts of the manuscript under Dr. Coussens' direct supervision.

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5. Sounni NE, Dehne K, vanKempen LCL, Egeblad M, Affara NI, Cuevas I, Wiesen J, Junankar S, Korets L, Lee J, Shen J, Morrison C, Overall CM, Krane SM, Werb Z, Boudreau N, **Coussens LM**. (2010) Stromal regulation of vessel stability by MMP14 and TGFβ. *Disease Model. Mech.* 3:317-332.

**Role:** In patients with locally advanced solid tumors, first-line treatment is often neo-adjuvant or pre-operative chemotherapy, which helps shrink tumors before surgery, allowing for more conservative surgical approaches and reducing the potential for developing systemic disease. However, despite aggressive chemotherapy, long-term survival for many patients remains poor, in part owing to limitations with the targeting and accumulation of cytotoxic drugs in tumor tissue. The vasculature of solid tumors is abnormal, both in terms of vessel architecture and the dynamics of blood flow. Permeable heterogeneous vessel walls allow the leakage of proteins and fluid that, coupled with the inefficiency of lymphatic drainage, could be exploited to develop novel,

enhanced drug delivery strategies that are therapeutically selective and improve clinical outcome. This work describes a previously unappreciated role for transforming growth factor beta (TGFβ) in regulating vascular stability and vessel permeability in solid tumors. Using mouse models, we demonstrated an endogenous pathway that regulates normal vascular permeability, which is controlled by perivascular collagen, the metalloproteinase enzyme MMP14, and TGFβ. In wild-type mice, inhibitors of either MMP14 or TGFβ signaling induce blood vessel permeability. Conversely, enhanced MMP14 or TGFβ activity in the mouse epidermis decreases leakage across cutaneous vessels. This pathway remains functional during tumor progression, as acute blockade of either MMP14 or TGFβ signaling transiently alters vessel stability, 'opening' vascular beds and promoting intravenous delivery of high molecular weight compounds to the tumor. This implying that delivery of standard therapeutic agents or diagnostic molecular imaging agents to tumor tissue may be enhanced by transient blockade of the TGFβ pathway. If so, this could advance disease therapy and/or diagnostic imaging, not only in cancer medicine, but also in fibrotic disorders such as scleroderma and kidney failure. Research in support of this manuscript spanned over 7 years and many post doctoral fellows and technicians. The initial hypothesis and ideas towards successful completion of the project were conceived by Dr. Coussens, who also wrote the manuscript with input from Drs. Boudreau and Werb.

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# Interactions between lymphocytes and myeloid cells regulate pro- versus anti-tumor immunity

**David G. DeNardo · Pauline Andreu · Lisa M. Coussens**

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**Abstract** Tumor-associated myeloid cells have been implicated in regulating many of the "hallmarks of cancer" and thus fostering solid tumor development and metastasis. However, the same innate leukocytes also participate in anti-tumor immunity and restraint of malignant disease. While many factors regulate the propensity of myeloid cells to promote or repress cancerous growths, polarized adaptive immune responses by B and T lymphocytes have been identified as regulators of many aspects of myeloid cell biology by specifically regulating their functional capabilities. Here, we detail the diversity of heterogeneous B and T lymphocyte populations and their impacts on solid tumor development through their abilities to regulate myeloid cell function in solid tumors.

**Keywords** Cancer · Inflammation · Lymphocyte · Macrophage · Metastasis

## 1 Introduction

Virchow first described leukocyte infiltration of solid tumors in the 1800s; however, only recently have we begun to understand the diverse regulatory roles played by immune cells during cancer development. Historically, leukocytes found in and around developing tumors were thought to represent an attempt by the host to eradicate neoplastic cells. Indeed, some leukocytes, including $CD8^+$ cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, do play a critical role in restraining tumor development [1]. However, we now appreciate that the significance of these anti-tumor programs can be thwarted by other subsets of leukocytes that instead foster tumor development [2–5]. Immune-competent mouse models of human cancer have enabled a detailed evaluation of the tumor-promoting capacity of several subsets of myeloid cells, including mast cells (MCs), monocytes, granulocytes/neutrophils, and macrophages, as well as some subsets of lymphocytes [6, 7]. However, depending on their differentiation status and immune microenvironment, subpopulations of these same cells can also support tumor rejection and response to anti-cancer therapy [2, 8, 9], thus indicating that pro- and anti-tumor programming of leukocytes is dynamic. In this review, we discuss recent insights into the role of B and T lymphocytes as "gatekeepers" of myeloid cell bioactivity (Fig. 1) and how recognition of these dynamic interactions reveals novel opportunities for anti-cancer therapy.

## 2 Paradoxical role of $CD4^+$ T lymphocytes in solid tumor development

In contrast to $CD8^+$ CTLs that play well-defined roles in hindering cancer development, the functional significance of $CD4^+$ T lymphocytes in tumor progression appears more paradoxical. For example, retrospective evaluation of colon and lung carcinomas revealed that extensive infiltration of tumors by $CD4^+$ T cells correlates with favorable clinical outcome, whereas in breast and renal cancers exhibiting similar infiltrations instead correlates with decreased overall

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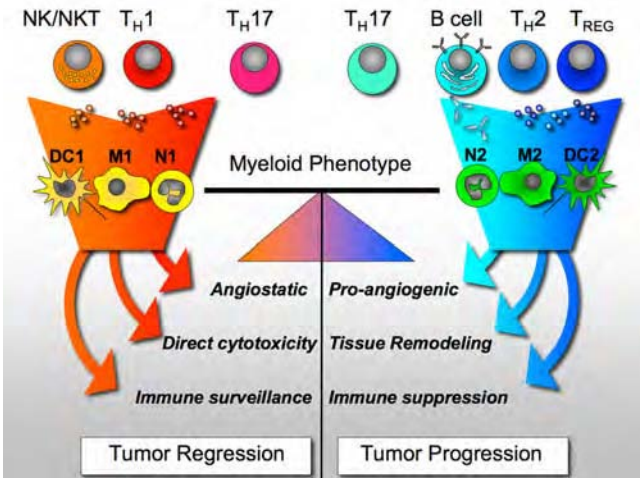


**Fig. 1** Adaptive immune responses control tumor-associated myeloid cell bioactivity and tumor progression. Polarized responses by adaptive immune cells alter the balance between pro- and anti-tumor myeloid cell bioactivities. When the host's response to neoplastic cell growth results in the production of $T_H1$ cytokines by $CD4^+$ T lymphocytes and NK cells, myeloid cells in turn induce programs promoting tumor regression and/or dormant disease. However, when these adaptive immune responses include chronic B lymphocyte activation and IgG production in combination with $T_H2$ and $T_{REG}$ lymphocyte activation, programs of immune suppression, angiogenesis, tissue remodeling, and invasion are favored in myeloid cells and contribute to tumor progression and metastasis

survival [10–13]. Analysis of mouse models of human cancer have provided some clarity for these disparate findings and revealed that etiology and organ specificity matters with regards to how $CD4^+$ T cells aid or constrain tumor progression. Schreiber and colleagues demonstrated that, whereas $CD4^+$ T cell deficiency enhances methylcholanthrene (MCA)-initiated sarcoma development [14], carcinoma development on the other hand is inhibited following two-stage squamous carcinogenesis [15, 16]. Similarly, in a mouse model of skin and cervical carcinoma development where oncogenes from human papilloma virus type 16 (HPV16) are expressed under the control of the keratin 14 promoter, skin carcinoma formation is modestly attenuated by $CD4^+$ T cell deficiency, whereas cervical carcinoma development is significantly enhanced [17, 18]. Together, these observations demonstrate that immune responses accompanying tumor development are organ dependent and, based on the neoplastic microenvironment, engage either pro- or anti-tumor immune programs. The heterogeneity of $CD4^+$ T cell subsets that accumulate in tissues may be at the heart of these paradoxical findings.

## 3 $CD4^+$ T lymphocyte heterogeneity

$CD4^+$ T cells represent a highly heterogeneous population of cells that develop along different functional lineages depending upon polarizing cytokine signals during activation by antigens [19]. Classically, $CD4^+$ T lymphocyte subsets include $T_H1$ and $T_H2$ lineages that are characteristically fostered by exposure to interleukin (IL)12 and IL4, respectively. Following activation, $CD4^+$ T cells assuming the $T_H1$ fate secrete interferon (IFN)γ, tumor necrosis factor (TNF)α, IL2, and IL12 [20]. Through the production of these cytokines, $T_H1$ cells regulate immune surveillance programs by up-regulating antigen processing and presentation on major histocompatability complex (MHC) I and II molecules by professional antigen presenting cells (APCs) and as such can regulate the duration and magnitude of $CD8^+$ CTL responses [21]. In addition, following strong antigen-specific activation, $T_H1$ cells can directly kill tumor cells by releasing high levels of IFNγ, TNFα and cytolytic granules. Thus, when present, $T_H1$ responses can directly and indirectly regulate anti-tumor programs that restrain cancer development.

In contrast, $T_H2$ $CD4^+$ T cells express high levels of IL4, -5, -6, -10, and -13 that, together, alter adaptive immunity by inducing T cell anergy, inhibiting T cell-mediated cytotoxicity as well as fostering humoral immune responses directed by B cells [22, 23]. The $T_H2$ cytokines IL4 and IL13 are important mediators of $CD4^+$ T cell functionality *in vitro*, $T_H2$ $CD4^+$ T cells inhibit apoptosis and induce proliferation of breast carcinoma cells; *in vivo*, IL4 emanating from $CD4^+$ T cells fosters breast cancer growth [24, 25]. Consistent with these findings, a high ratio of $T_H2^+$ to $T_H1^+$ cells correlates with parameters of clinical disease progression, such as increased tumor size and grade and lymph node metastasis of breast cancers [26].

Adding to this $T_H1$ versus $T_H2$ paradigm, CD4 lineages have recently expanded to include a $T_H17$ subset that is differentiated by a combination of IL6 and transforming growth factor (TGF)β and mediate their effects through secretion of IL17, -21, and -22, [27–29]. $T_H17$ cells are thought to play an important role in protection against some extracellular pathogens and in regulating auto-immune disease [30, 31]. As such, $T_H17$ cells have been implicated in the development of inflammation-associated colonic tumors in response to pathogenic bacteria [32]. $T_H17$ cell infiltration has also been observed in patients with colon, ovarian, prostate, and hepatocellular carcinoma where high numbers of IL17-producing cells correlates with poor prognosis [33–35]. In mouse models of non-small cell lung cancer (NSCLC), IL17 enhanced tumor growth by promoting development of angiogenic vasculature [36, 37]. In contrast, in a B16 melanoma model, IL17 depletion rendered mice more susceptible to metastasis [38], a phenotype that was blocked by adoptive transfer of tumor-specific $T_H17$ cells that fostered immune surveillance by $CD8^+$ CTLs and dendritic cells (DCs) [38]. Together, these experimental findings indicate that the role

of $T_H17$ cells in regulating aspects of cancer development may also be context dependent.

In addition to $T_H1$, $T_H2$, and $T_H17$ $CD4^+$ effectors, populations of $CD4^+FoxP3^+$ T regulatory ($T_{REG}$) cells are also thought to play a considerable role in regulating tumor immunity. In human cancers, increased prevalence of $CD4^+FoxP3^+$ $T_{REG}$ cells correlates with increased survival for follicular lymphoma [39], while it instead correlates with poor prognosis in pancreatic ductal carcinoma [40], non-small cell lung cancer [41], renal cell carcinoma [11], and breast carcinoma [42]. Suppression of the anti-tumor activities of $CD8^+$ CTLs, NKs, and DCs is at the heart of how $T_{REG}$ cells control tumor development [43, 44]; however, the multitude of mechanisms whereby they regulate anti-tumor programs suggest the existence of distinct tissue-specific subpopulations of $T_{REG}$ cells, each endowed with or capable of various bioeffector activities [45–47]. Mechanistic studies have revealed that $T_{REG}$ cells support pro-tumor immunity not only by increasing local levels of immunosuppressive cytokines including TGFβ, IL35, and IL10 but also by direct cytolytic effects through production of perforin and granzyme. In addition, $T_{REG}$ cells can disrupt the metabolic activity of cyclic adenosine monophosphate (cAMP) transfer, as well as inhibit APC function by inducing binding of CTLA-4 to CD80/86 [44, 48].

Thus, while it is now clear that a spectrum of $CD4^+$ T cell subtypes are present in human tumors of essentially all types, the role they play in promoting or repressing tumor development likely has to do with the type of $CD4^+$ T cell subtype that is either recruited to or accumulates within each distinct tumor microenvironment. These in turn then regulate anti-tumor programs by professional cytotoxic cells ($CD8^+$ T CTLs and NK cells), as well as regulating pro-tumor properties of a diverse array of myeloid cell subtypes as discussed below.

## 4 Myeloid heterogeneity and tumor development

Innate immune cells of myeloid origin, e.g., granulocytes (neutrophils, basophils, and eosinophils), DCs, macrophages, NK cells, and MCs, are also prominent components of pre- and malignant tissues and functionally contribute to cancer development by releasing a myriad of cytokines, chemokines, matrix metalloproteinases, serine proteases, DNA-damaging molecules (reactive oxygen species), histamine, and other bioactive mediators that regulate tissue remodelling and angiogenesis [49–53], suppress anti-tumor immunity [54–56], and enhance tumor cell survival, migration, and metastasis [57, 58].

Nucleated hematopoietic cells that have been directly implicated in tumor angiogenesis include MCs [51], tumor-associated macrophages (TAMs) [5, 23, 59], Tie2-expressing monocytes [50, 60], neutrophils [52], DC precursors [61], and myeloid immune suppressor cells [62, 63]. Other hematopoietic cell types, such as platelets [64], eosinophils [65], and hematopoietic progenitors [66], also participate in angiogenic processes, but it remains to be established whether they can directly promote tumor angiogenesis, rather than having a broader function in supporting tissue inflammation and remodelling.

In contrast, these same myeloid cell lineages also foster tumor rejection by inducing angiostatic programs, enhancing CTL and NK responses, and directly inducing tumor cell death [13]. As an example of these paradoxical roles, studies from several laboratories have reported that TAMs enhance angiogenesis and metastasis of malignant mammary tumors [25, 67, 68]. In contrast, TAMs exposed to toll-like receptor (TLR) ligands and/or IFNγ directly lyse mammary tumor cells, increase antigen presentation, and secrete angiostatic proteins such as CXCL10 and 11 [8, 9 , 69]. These distinct bioactivities are mirrored in neutrophils, MCs, and DCs and may be due to the inherent plasticity of myeloid lineage cells regulated by local factors present in distinct tissue and/or organ microenvironments.

The bioactive states of macrophages, as well as other myeloid cells, have been classified according to $T_H1$ and $T_H2$ nomenclature, referred to as M1 (classical) or M2 (alternative) activation, respectively [2, 70, 71] (Fig. 1). M1 macrophages are regulated by $T_H1$ cytokines including IFNγ, TNFα, and granulocyte monocyte-colony stimulating factor (GM-CSF) that enhance macrophage cytotoxic activity, production of pro-inflammatory cytokines, and antigen presentation capacity [70, 71]. In contrast, tissue macrophages can achieve various alternatively activated M2 states following exposure to $T_H2$ cytokines, including IL4 or IL13 (M2a), potentiation by immune complexes and TLR ligands (M2b) or immunosuppressive cytokines including IL10 or TGFβ and/or glucocorticoid hormones (M2c) [70].

The general hallmarks of M2 macrophages include high levels of IL10, IL1Ra, IL1 decoy receptor CCL17 and CCL22 secretion, high expression of mannose, scavenger and galactose-type receptors, low expression of IL12, as well as poor APC capability. Intriguingly, although these alternative activation states (M2a, b, and c) share many phenotypic characteristics, they are distinct and induce individual context-dependent environmental responses. For example, induction of an M2c phenotype by IL10 results in highly immune suppressive macrophages that can also produce matrix components such as versican or PTX3. In contrast, $T_H2$ cytokine induction of M2a TAMs induces expression of fibronectin, as well as catabolism of *L*-arginine by arginase that in turn leads to increased collagen synthesis and matrix remodeling [70, 72]. Our own work has revealed that IL4 and/or IL13 activation of macro-

phages induces production of growth factors including epidermal growth factor, TGFβ, and basic fibroblast growth factor that together regulate invasive, angiogenic, and immune-suppressive programs [25] (unpublished data). Both M2a and M2b macrophages down-regulate the pro-inflammatory cytokines IL1, IL6, and TNFα [73], whereas induction of M2b macrophages by immune complexes induces these same inflammatory cytokines in addition to IL10 and also likely enhances vascular responses such as endothelial migration and vessel dilation [74]. Thus, M2-polarized cells promote scavenging of debris, angiogenesis, and remodeling and repair of wounded/damaged tissues. Parallel and non-redundant activity states have also been defined for DCs (i.e., DC1, DC2) [75] and neutrophils (i.e., N1, N2) [76] (Fig. 1).

To address pro- versus anti-tumor capabilities of these opposing states, Hagemann and colleagues demonstrated that by "reprogramming" M2 TAMs through deletion of IKKβ macrophage phenotype could be switched from immunosuppressive to actively promoting immune surveillance, as reflected by decreases in IL10 and arginase-1 expression and increased IL12 and nitric oxide production together resulting in decreased ovarian tumor growth through recruitment and activation of NK cells [67, 77]. Again, organ specificity and/or etiology may play a role in regulating how reprogramming can be achieved. In a mouse model of squamous carcinogenesis, we recently reported that FcγR signaling in myeloid cells directly regulates whether myeloid cells enhance or repress cancer development, which correlate with their unique gene expression signatures that reflect M1 versus M2 and DC1 versus DC2 programs [74]. These data indicate the significance of reprogramming myeloid cell phenotypes to affect tumor outcome. The major question that arises with regards to this capability then becomes what are the cellular and molecular programs in tissues and/or tumors that regulate the bioactive state of these important myeloid cells and how recognition of these can be translated into anti-cancer therapy.

## 5 T lymphocytes as regulators of anti-tumor macrophages

Establishment of an immune reaction in homeostatic tissue typically involves activation of NK cells in response to stress signals or infectious agents, whom by their production of IFNγ in turn prime macrophages towards an M1 state, culminating in enhanced presence of macrophages with cytotoxic capability [71]. However, production of IFNγ by NK cells is generally transient and therefore insufficient to sustain M1 macrophage polarization; thus, IFNγ-producing $T_H1$ cells are critical for immune responses requiring sustained M1 macrophage bioactivities. In tumors, studies by Corthay and colleagues demonstrated that $T_H1$ cell regulation of locally activated M1 macrophages were significant and fostered rejection of myeloma and lymphomas in the absence of CTL responses [69, 78]. Moreover, expression of IL12 and IFNγ by $T_H1$ cells can combine to enhance anti-tumor responses by NK and NK-T cells by up-regulating expression of NK receptors such as NKG2D (in response to IL12) and expression of NK receptor ligands such as RAE1 on target cells (in response to IFNγ) [79]. $T_H1$ responses then in turn favor anti-tumor NK and macrophage responses that eliminate neoplastic cells. However, while $T_H1$ cells are antigen specific, tumoricidal macrophages exert indiscriminate cell killing activity. Thus, multiple immunosuppressive programs have evolved to eliminate the adverse autoimmune pathologies, such as rheumatoid arthritis, that are associated with over-activation of these M1 responses [80]; unfortunately, many of these immunosuppressive programs are usurped by developing cancers.

## 6 T lymphocytes as regulators of pro-tumor myeloid cells

In contrast to induction of tumor-immune surveillance programs by $T_H1$ cells, $T_{REG}$ and $T_H2$ cells have the capacity to induce alternative activation states of macrophages, DCs, and neutrophils involved in promoting cancer development. Studies of human $T_{REGs}$ have demonstrated their ability to block classical activation of macrophages and instead foster immunosuppressive myeloid phenotypes through the production of IL10 and TGFβ [81]. Similar biology may apply to neutrophils, as recently reported by Fridlender and colleagues who found that loss of TGFβ signaling through ALK4/5 inhibition resulted in recruitment of N1-polarized neutrophils with tumoricidal bioactivities [76]. While TGFβ in the tumor microenvironment is produced by multiple cell types, these data may indicate that $T_{REG}$ cells suppress N1 tumoricidal responses through production of TGFβ and, as such, favor pro-tumor N2 or immature monocyte (IMC) phenotypes. Our own work has demonstrated that $CD4^+$ $T_H2$ cells in mammary tumors promote M2 responses in TAM and IMCs that in turn enhance pulmonary metastasis [25]. Together, these data indicate that the balance of $T_H1$ versus $T_H2/T_{REG}$ responses regulates the pro- versus anti-tumor programming of tumor-associated myeloid cells.

## 7 B lymphocytes as regulators of myeloid cells during cancer development

B lymphocytes constitute a central component of humoral immunity and not only serve in antibody production but also in antigen presentation and cytokine secretion. In

particular, B lymphocyte expression of MHC and co-stimulatory molecules as well as secretion of pro-inflammatory cytokines are critical for regulating $CD4^+$ and $CD8^+$ T cell activation, expansion, antigenic spreading, and memory T lymphocyte formation. The heterogeneity of B lymphocyte responses has been recently recognized and diverse B cell subtypes with either pro-immune or regulatory properties have been identified *in vivo*. Precisely, regulatory B lymphocytes ($B_{REG}$), which include various subtypes of IL10-producing cells, have been identified in the context of autoimmune diseases and exert anti-inflammatory functions [82, 83]. However, a role for $B_{REG}$ cells in cancer has not been fully elucidated.

B lymphocytes in general have only recently gained recognition for representing significant components of tumor immunity [84]. B cell involvement in solid tumor development was initially described in syngeneic allograft murine tumor models in combination with genetic or antibody-mediated B cell depletion. In these studies, B cell-deficient mice (μMT) exhibited resistance to several types of syngeneic tumors, including EL4 thymoma, MC38 colon carcinoma, and B16 and D5 melanoma [85, 86], whereas partial B cell depletion resulted in significantly reduced tumor burden in a transplantable model of colorectal cancer [87]. A tumor-promoting role for B cells in solid tumor development was also revealed in transgenic mice expressing tumor necrosis factor (TNF)-receptor-associated factor 3 (TRAF3) in lymphocytes [88]. $TRAF3^+$ lymphocytes induce humoral immune responses leading to chronic inflammation and a significantly elevated incidence of squamous cell carcinomas [88]. These experimental findings indicate that, in the absence of an initiating oncogenic event, B lymphocyte-mediated chronic inflammation is sufficient to foster solid tumor formation. In contrast, an opposite and anti-tumor immune surveillance role for B lymphocytes has also been demonstrated in a syngeneic melanoma model where deletion of mature B cells by anti-CD20 IgG significantly enhanced tumor growth and metastasis [80], suggesting that the role of B lymphocytes in tumor progression, like $CD4^+$ T lymphocytes, may be context dependent and driven by individual B lymphocyte subtype specificity.

Mechanistically, B cells and humoral immunity can act to modulate solid tumor development by regulation of diverse effector pathways involving secretion of pro-inflammatory, as opposed to regulatory, cytokines, e.g., IL10, TGFβ, inhibition of CTL activity [89], perturbation of $T_H1/T_H2$ $CD4^+$ T cell lineages [90, 91], as well as differential recruitment and activation of innate immune cells [89, 92].

Recently, using a transgenic mouse model of inflammation-associated carcinogenesis, i.e., K14-HPV16 mice [93], we revealed a novel pathway by which B lymphocytes enhance squamous carcinogenesis and demonstrated the significance of the B cell/immunoglobulin/FcγR signaling axis. We found that B cells and humoral immunity fostered cancer development by activating Fcγ receptors on resident and recruited myeloid cells [74]. Stromal accumulation of autoantibodies in premalignant skin, through their interaction with activating FcγRs, regulated recruitment, composition, and bioeffector functions of leukocytes, in particular subsets of tumor-promoting polarized myeloid cells in neoplastic tissue which in turn enhanced neoplastic progression and subsequent carcinoma development [74]. A similar pro-tumor role for B cells was recently reported by Ammirante and colleagues who found that B cells are critical for growth of castration-resistant prostate cancer metastasis, not through production of immunoglobulins or regulation of FcγR signaling but instead by delivery of lymphotoxin that in turn activates IKK-α and STAT3 in prostate cancer cells and subsequently stimulates metastasis by an NF-κB-independent, cell-autonomous mechanism [94]. These findings together with other experimental studies support a model in which B cells, through various mechanisms, including humoral immunity, activating FcγRs and IKK, are required for establishing chronic inflammatory programs that promote *de novo* carcinogenesis.

## 8 Conclusions

While many factors regulate the propensity of immune cells to promote or repress solid tumor development, polarized adaptive immune responses by B and T lymphocytes can specifically regulate multiple pro-tumor properties of myeloid cells that in turn control many of the "hallmarks" of cancer development [95, 96]. Thus, recognition of the soluble molecules that mediate these important paracrine interactions may represent critical targets to evaluate for anti-cancer therapy. Importantly, targeting of pro-tumor pathways that neutralize M2-type macrophage and/or $T_H2$-type $CD4^+$ T cell responses and therein foster M1 or $T_H1$-type immunity may enhance sensitivity to cytotoxic therapies, including chemo- and radiation therapy, whose durability may be limited by the longevity of the anti-tumor immune responses that they induce.

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**LISA M. COUSSENS, PH.D.**

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## REGULATION OF PROTUMOR IMMUNITY AND CANCER DEVELOPMENT

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Clinical and experimental studies have established that chronic infiltration of neoplastic tissue by leukocytes, i.e., chronic inflammation, promotes development and/or progression of various solid tumors[1,2]; however, the organ-specific cellular and molecular programs that favor pro-tumor, as opposed to anti-tumor immunity by leukocytes are incompletely understood. While some leukocytes certainly exhibit anti-tumor activity, i.e., cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells[3], other leukocytes, most notably mast cells, $CD4^+$ T cells, B lymphocytes, dendritic cells (DCs), granulocytes, immature monocytes and macrophages exhibit more bipolar roles, by virtue of their capacity to either hinder or potentiate tumor progression[1,2]. A major question regarding these disparate leukocytes bioactivities is the degree to which their various pro- or anti-cancer activities are regulated by tissue-specificity and/or are responsive to individual oncogenic or tumor suppressive gene programming in early neoplastic tissue.

***Leukocytes and Breast Carcinogenesis***: In the breast, cancer development is in part characterized by a significant increase in both innate and adaptive immune cells, with B and T lymphocytes and macrophages representing the most abundant leukocytes present in neoplastic stroma[4]. Retrospective clinical studies examining identity of leukocytes in human breast cancer have revealed that high immunoglobulin (Ig) levels in tumor stoma (and serum), and increased presence of extra follicular B cells, T regulatory ($T_{reg}$) cells, high ratios of CD4/CD8 or $T_H2/T_H1$ T lymphocytes in primary tumors or in draining lymph nodes correlates with tumor grade, stage and overall patient survival[3]. On the other hand, experimental studies have demonstrated that macrophages in primary mammary adenocarcinomas regulate late-stage carcinogenesis by virtue of their pro-angiogenic properties[5,6], as well as fostering pulmonary metastasis by providing epidermal growth factor (EGF) and cathepsin proteases[7] to malignant mammary epithelial cells (MECs) and thereby enhancing their invasive (and metastatic) behavior[8,9]. Based on these seemingly disparate observations, we sought to determine if adaptive immunity also fostered malignancy in the breast by regulating the phenotype or effector functions of tumor-associated macrophages (TAMs) and either activated their pro-tumor properties or alternatively by suppressing their anti-tumor capabilities.

Utilizing the MMTV-PyMT mouse model of mammary carcinogenesis[10], we revealed a provocative role for $CD4^+$ T cells as potentiators of peripheral blood dissemination and pulmonary metastasis of malignant mammary adenocarcinomas through their ability to regulate pro-tumor properties of TAMs[11]. Specifically, $T_H2$-polarized $CD4^+$ T cells secrete high levels of interleukin (IL)-4 and thereby regulate M1 and M2-type TAM bioactivity by activation of IL4R$\alpha$-signaling cascades. M2-TAMs in turn promote invasive behavior of malignant MECs by high level production of cathepsin protease activity[7] and EGF that subsequently activates MEC invasion and EGF receptor signaling programs, activities that are essential for entry into peripheral blood, dissemination and outgrowth in the lung. These findings indicate that when $CD4^+$ T lymphocytes are present in a $T_H2$-type tumor microenvironment, they promote metastasis by regulating the pro-tumor properties of TAMs mediated by IL4R$\alpha$-signaling, as opposed to limiting or eradicating malignant cells by engaging cytotoxic mechanisms. These realizations provide rational for development of anti-cancer therapeutics that neutralize pro-tumor properties of IL4R$\alpha$-based signaling in both adaptive and innate immune cells in the tumor microenvironment and periphery, that when delivered in combination with cytotoxic drugs or therapeutics bolstering anti-tumor immunity, may extend survival of patients with advanced disease.

***Leukocytes and Squamous Carcinogenesis***: B lymphocytes constitute a central component of adaptive immunity and not only serve in antibody production but also as antigen-presenting cell; thus, B lymphocyte expression of major histocompatability complex (MHC) and co-stimulatory molecules as well as secretion of pro-inflammatory cytokines induces optimal $CD4^+$ and $CD8^+$ T cells activation,

expansion, memory T lymphocyte formation and antigenic spreading. As such, B cells have been historically associated with anti-tumor immunity. More recently, the heterogeneity of B lymphocyte responses has been recognized and diverse B cell subtypes with either pro-immune or regulatory properties have been identified *in vivo*. In particular, regulatory B lymphocytes, which include various flavors of IL-10 producing cells, have been identified in the context of autoimmune diseases that exert anti-inflammatory activities[12,13]. However, the role of these individual B lymphocyte subpopulations in malignant disease has yet to be fully elucidated.

Using a transgenic mouse model of multi-stage epithelial carcinogenesis, *i.e.*, K14-HPV16 mice[14], we previously revealed that adaptive immunity is an important regulator of inflammation-associated cancer development[15]. Combined B and T lymphocyte-deficiency in HPV16 mice, *e.g*. HPV16/RAG1$^{-/-}$ mice, resulted in a failure to initiate and/or sustain leukocyte infiltration during premalignancy[15]. As a consequence, tissue remodeling, angiogenesis and epithelial hyperproliferation were significantly reduced, culminating in attenuated premalignant progression and a 43% reduction in carcinoma incidence[15]. Importantly, adoptive transfer of B lymphocytes or serum from HPV16 mice into HPV16/RAG1$^{-/-}$ mice reinstated chronic inflammation in premalignant tissues, indicating that B cell-derived soluble mediators were necessary to potentiate malignant progression. More recently, we revealed that B cell-derived IgGs regulate neoplastic progression and subsequent carcinoma development by engagement of Fc$\gamma$ receptors (Fc$\gamma$R) expressed on resident and recruited immune cells[16]. Specifically, we found that immune complex (IC)-stimulation of leukocyte FcR$\gamma$ is critical for establishing a pro-tumor microenvironment in premalignant tissue that directs not only recruitment of leukocytes from peripheral blood, but also leukocyte composition, phenotype and bioeffector functions once within neoplastic tissue. As such, proangiogenic and protumorigenic functions of mast cells and macrophages are differentially regulated by humoral immunity and functionally contribute to squamous carcinogenesis. These findings have broad clinical implications as they reveal critical signaling pathways regulated by humoral immunity and FcR$\gamma$ to target therapeutically in patients suffering from chronic inflammatory diseases at risk for cancer, as well as individuals harboring premalignant lesions where chronic inflammation compromises tissue integrity and enhances risk of malignancy.

***Summary***: While many factors regulate leukocyte propensity to either promote or repress tumor development, in tissue- and/or oncogene-specific manners, polarized $T_H2$-type adaptive immune responses foster pro- as opposed to anti- tumor programming of myeloid cells that in turn directly regulate many of the "hallmarks" of solid tumor development[17]. Neutralizing these various "pro-tumor" regulatory pathways may provide relief for some aspects of late-stage cancer development as monotherapy, but more likely when combined with cytotoxic-, targeted- and/or immuno-therapy will provide a survival advantage by bolstering induction of anti-tumor bioactivities of tumor-associated leukocytes that extend efficacy of therapy.

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**LISA M. COUSSENS, PH.D.**

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## REGULATION OF PROTUMOR IMMUNITY AND CANCER DEVELOPMENT

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*Summary*: While many factors regulate the propensity of leukocytes to promote or repress primary tumor development and metastasis, some of which may be tissue- and/or oncogene-specific, polarized $T_H2$-type adaptive immune responses by B and T lymphocytes foster pro- as opposed to anti- tumor programming of myeloid cells that in turn directly regulate many of the "hallmarks" of solid tumor development [24]. Neutralizing these various "pro-tumor" regulatory pathways may provide relief for some aspects of late-stage cancer development as monotherapy, but more likely when combined with cytotoxic-, targeted- and/or immuno-therapy will provide a survival advantage by bolstering induction of anti-tumor bioactivities of tumor-associated leukocytes that extend efficacy of therapy.

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# Inflammation and Cancer:

## Polarized Immune Responses Regulate Cancer Development

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The concept that leukocytes are components of malignant tumors is not new; however, their functional involvement as promoting forces for tumor progression has only recently been appreciated. We are interested in understanding the molecular mechanisms that regulate leukocyte recruitment into neoplastic tissue and subsequent regulation those leukocytes exert on evolving cancer cells. By studying transgenic mouse models of skin, lung and breast cancer development, we have recently appreciated that adaptive leukocytes differentially regulate myeloid cell recruitment, activation, and behavior, by organ-dependent mechanisms. Thus, whereas premalignant progression, including chronic inflammation, activation of angiogenic programming, tissue remodeling and malignant conversion during skin carcinogenesis is B cell, Ig and Fc$\gamma$R–dependent, during mammary carcinogenesis by contrast, $T_H2$-polarized $CD4^+$ T cells play a dominant role in regulating pro-tumor and pro-metastatic properties of M2-polarized macrophages and dendritic cells, that together regulate metastasis of malignant mammary epithelial cells to lung. To be presented will be recent insights into organ and tissue-specific regulation of epithelial cancer development by adaptive and innate immune cells, and thoughts on how these properties can be harnessed for effective anticancer therapeutics.

Funding from the NIH/NCI and Department of Defense Era of Hope Scholar Award and Program in Mesothelioma

**Oral Presentation at the 2010 Annual Meeting of the American Association for Cancer Research (AACR), Washington DC, USA.**

## Targeting Macrophages as a Novel Therapeutic Approach for Malignant Pleural Mesothelioma

Nikita Kolhatkar[1,3], Magnus Johansson[1], Dario Barbone[3], Adam Yagui-Beltrán[2], Raphael Bueno[4], David J. Sugarbaker[4], David M Jablons[2,5], V. Courtney Broaddus[3], Lisa M Coussens[1,5]

Departments of [1]Pathology, [2]Surgery, [3]Lung Biology Center, and [5]Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco. [4]Division of Thoracic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts.

Mesothelioma is a life-threatening tumor, induced by inhalation of asbestos fibers, which is largely resistant to most chemotherapeutic approaches. One feasible approach could be to harness the power of the immune system to increase the chemosensitivity of mesotheliomas. Using a combination of immunohistochemistry and flow cytometry to analyze the leukocyte compositions of human mesotheliomas, we have found that 1) epithelioid and mixed mesothelioma tumor subtypes have a higher degree of immune cell Infiltration, when compared to sarcomatous tumors, and 2) mesothelioma tumors have large infiltrations of macrophages (31 ± 4.6% of the inflammatory cell population [$CD45^+$]). Indeed, the percentage of macrophages in mesotheliomas exceeded that found in other thoracic malignancies thus far evaluated (NSCLC cancer, 9%; esophageal, 4%). In view of recent data indicating that macrophages can be targeted therapeutically to minimize some aspects of cancer development, we investigated whether macrophages could be targeted to enhance chemosensitivity of human mesotheliomas. To address this question, we adapted a 3-dimensional spheroid growth model, enabling heterotypic culture of mesothelioma cells with macrophages. We found that mesothelioma chemoresistance can be lowered by co-incubation with macrophages. However, the magnitude of the response was dictated by specific macrophage phenotype. Macrophage phenotype and bioactivity is modulated by Th1 versus Th2 cytokine exposure that in turn regulate either an M1 (IFN-γ & LPS) or M2 (IL-4) phenotype. M1-polarized macrophages increased the response of malignant mesothelioma spheroids to pro-apoptotic chemotherapeutic agents, such as TRAIL and gemcitabine. Furthermore, our preliminary data indicate that primary human tumor-associated macrophages, isolated from untreated malignant mesotheliomas, have similar pro-apoptotic effects when polarized with M1 cytokines, suggesting that cytokine re-polarization of macrophages in mesothelioma tumors to an M1 phenotype could augment therapeutic efficacy.

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